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Bioreactor Design for Vascular Tissue Engineering

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BIOREACTOR DESIGN FOR VASCULAR TISSUE ENGINEERING

A Major Qualifying Project Report

Submitted to the Faculty

Of the

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

by

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Date: April 30, 2009

Approved:

1. Bioreactor

2. Blood Vessels

3. Tissue Engineering

Prof. Marsha Rolle, PhD, Major Advisor

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Authorship

All four team members contributed to and reviewed all sections of this report and therefore we jointly accept responsibility for the project and decline the option of individual authorships.

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Abstract

Currently, there is an urgent clinical need for blood vessel replacements, especially in small diameter applications. Tissue engineered vascular grafts take months to culture and require extensive graft manipulation. The purpose of this project was to design a bioreactor which would output small diameter tubular and completely cellular vascular grafts. A cartridge was designed as a housing for five hollow, porous fibers used as support structures for the growth of cells seeded into the bioreactor. A pump was used to create a dynamic culture system and to facilitate cell seeding onto the fibers with the use of a vacuum force. The design was validated by analyzing the fluid flow and forces generated. Computer modeling and physical flow testing indicate that the bioreactor is suitable for cell seeding and vascular graft production. Future analysis of fiber materials will focus on more accurate modeling of fluid flow and assessment of cell seeding and vascular graft assembly.

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1.0 Introduction

Currently, there is an urgent clinical need for vascular grafts, especially for small diameter (<6mm) applications. Small diameter grafts are commonly used in arterial bypass surgeries and in 2005 alone, 600,000 arterial bypass surgeries were performed (Antman et. al., 2004). Additionally, small diameter vascular grafts are needed for dialysis fistulas and lower limb vessel replacements. The use of autologous grafts, tissues transplanted from one area in the human body to another, requires an additional surgery. Also, many patients lack suitable autologous vessels due to age, disease, or previous harvest (Schmelden et al., 2003). When suitable autologous vessels are unavailable, completely synthetic grafts have been used. Although these synthetic grafts may be suitable for large-diameter applications, synthetic grafts are highly susceptible to clotting when used for small diameter applications (Harbuzariu et al, 2007). There are three main categories for grafts including synthetic, autologous and tissue engineered.

A number of tissue engineered grafts have been explored, but currently there is not an abundance of available grafts. The majority of current tissue engineering approaches involves the use of exogenous scaffolds, made of synthetic polymers, protein hydrogels, crosslinked proteins, or decellularized native tissues (Stegemann et al, 2007). Cells seeded into these exogenous scaffolds are cultured *in vitro* until suitable biological and mechanical function is achieved. The scaffolds are intended to provide mechanical support and biochemical cues so that the cells will grow into functional vessels, but there are many difficulties encountered by using exogenous scaffolds. The use of exogenous scaffolds often leads to inconsistent degradation rates, compliance mismatch between the old and new vessels, and a lack of biocompatibility. Although exogenous scaffolds are not as elaborate as cell based grafts, the complexities of these scaffolds make them difficult to manufacture due to their size, materials and specific surface geometry. An alternative approach to using exogenous scaffolds involves stimulating the cells to self assemble their own scaffold or extra cellular matrix (ECM) (Sun et al, 2005). In order to create a tissue engineered vascular graft without complications common to exogenous scaffolds, a graft should be developed that is completely cell-derived.

One company, Cytograft, uses a sheet based technology to produce completely tissue engineered vascular grafts. The process begins once a skin biopsy is taken from the patient. The human dermal fibroblasts (HDFs) are isolated using collagenase digestion, and the cells are allowed to culture. The HDFs are grown into cell sheets, which take six-eight weeks to mature. Once the sheets have been grown, they are rolled four times around a stainless steel cylinder with an outer diameter of 4.75 mm. The sheets are then cultured for 12 weeks to allow the layers to fuse together while air drying in a tissue culture hood to form an acellular internal membrane. Additional living fibroblasts sheets are then wrapped around this structure four times and matured for an additional six-eight weeks. The resulting structures are then available for *in vivo* use. Currently these vessels are in clinical trials as dialysis fistulas (Konig, 2009) While the clinical trials are promising, this method is lengthy with an overall process totaling up to 28 weeks (McAllister, 2006).

While the method used to create vascular grafts is crucial, the system and bioreactor the tissue is grown in is also important. The state of the art for creating a completely cell-derived vascular graft requires manipulation of the tissue and is very time consuming. The current methods also use the traditional method of growing cells on a flat plate, where they grow and divide naturally with ease. Creating a bioreactor that can produce cylindrical vascular grafts could essentially remove manual rolling steps, therefore eliminating the time it takes to produce these grafts and contamination risks. The proposed bioreactor aims to culture cells to self-assemble into vascular grafts, a difficult change in itself. A bioreactor that is inexpensive to manufacture is also needed to save on the large costs spent on the research using blood vessels. The bioreactor has the potential to create grafts that could be used to eliminate painful surgeries associated with using autologous grafts as well as the complications associated with using synthetic grafts. Since the tissue engineered vessels use autologous cells, the biocompatibility risks may be minimized are irrelevant. With proper conditioning, the correct mechanical properties could be produced, unlike current technology with synthetic grafts.

Therefore, the goal of this project is to design, build and develop a method for a bioreactor to facilitate cell assembly into blood vessels without the use of exogenous scaffolds. The bioreactor should also minimize cell and tissue manipulation so that the

vessels can be produced both quickly and easily. The completely biological vascular grafts should be approximately 2mm in diameter, 5cm in length, and 250 μm in wall thickness, consequently the bioreactor should be designed to fulfill these requirements. Designing and building a bioreactor that could use a patient's own cells to produce multiple grafts would allow for testing of the grafts to make sure they are ready to be implanted. Since it is likely not all the grown vessels would be necessary for graft replacement, any additional could be used to test the properties. Multiple graft reproduction is also important for the research standpoint, where different aspects of the graft would be investigated requiring multiple grafts to be tested. The bioreactor should also be adaptable for different size specifications that may be required in the future.

In order to design and build a bioreactor there were certain steps that needed to be completed to verify that the design fits the desired outcomes the most effectively. The design method that was used was a five step process revised from *Engineering Design: A Project-Based Introduction* written by Clive Dym and Patrick Little. The steps were based on the initial client statement from which the problem definition, conceptual design, preliminary design, detailed design, and design communication led to the final design.

The first stage, the problem definition, took the client statement to first clarify the design objectives, establish the user requirements and functions and to identify the constraints. The revised client statement, objectives, constraints, user requirements and functions, which will be described later in Chapter 3.0, were established next. The design specifications and design alternatives were used to generate the conceptual designs, which had several aspects, including a mandrel to seed cells on, a method to seed the cells and a system to grow the cells in. During the preliminary design stage, which was the stage that a final design was chosen, testing and evaluation of the results were completed to determine which aspects would be included in the final design. During the fourth stage, the detailed design, the final design was refined based on testing and the proposed fabrication specifications were determined. Ultimately, the fifth and final stage was the design communication stage in which the design was documented and submitted to the client.

Prior to the design stages, related topics were researched in order to understand the client statement and define it more clearly. An in-depth patent search was also conducted in order to ensure that the design was original and no similar devise had been patented. Sections of the paper were also included to describe the effects the project may have on aspects such as the environment and the economy. Finally, future recommendations were made so that improvements can be made to the design in the future based on the results and conclusions that were determined.

2.0 Literature Review

Small-diameter graft replacements have become increasingly essential in recent years as hospital stays and the money spent on Coronary Heart Disease (CHD) has increased significantly (Shaw, 2006). Although synthetic and autologous grafts have proven to be adequate, there is a growing need for a completely cell derived small-diameter vascular grafts to minimize complications and reduce costs. Clinical trials have proven that completely cell derived small-diameter vascular grafts have minimal side effects and complications that have been common in patients with synthetic grafts (McAllister, 2006).

2.1 Coronary Heart Disease

Coronary Heart Disease, affecting the vasculature of the heart, was the cause of one out of every five deaths in the U.S. year 2005 (National Center for Health Statistics, 2005). Nearly 450,000 Americans died from the disease in the year 2005. A higher proportion of men have cardiovascular disease than women, one in six women have the disease. In 2005, the disease accounted for one out of every 2.7 deaths for women (National Center for Health Statistics, 1979-2005). While life style choices have been shown to increase or decrease the risk of developing coronary heart disease, many Americans also inherit genes that increase their risk (Stampfer et al, 2000).

An estimated 8 million Americans have had a myocardial infarction, out of a total of almost 17 million Americans that have been diagnosed with at least one type of coronary heart disease (Lloyd-Jones et al, 2009). Myocardial infarction is a common result of coronary heart disease. Infarct occurs when a blockage in the coronary artery cuts off blood flow to part of the heart. The resulting blockage can lead to the death of the distal heart tissue. In order to repair this condition, a common solution is to use a coronary artery bypass graft (CABG), which bypasses the blockage and restores blood flow to the affected tissue, as seen in Fig. 2.1. Small diameter vascular grafts are used in patients with coronary heart disease to bypass blockages in the coronary arteries and restore blood flow to the heart.

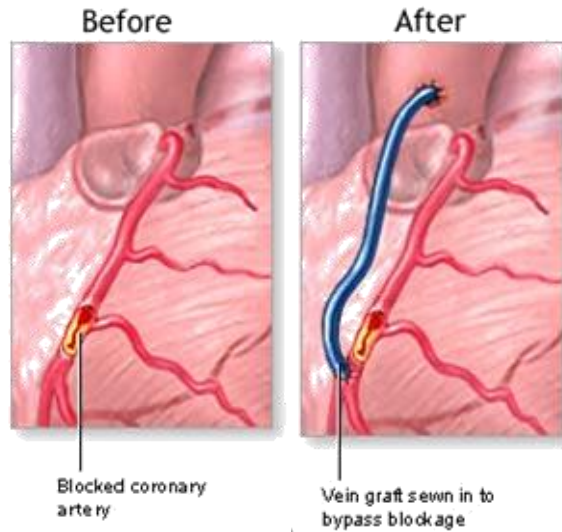


Figure 2.1: Coronary Artery Bypass Graft

Found at: http://www.nlm.nih.gov/medlineplus/ency/presentations/100190_1.htm

The American Heart Association estimated that in the year 2009, CHD would be responsible for over 750,000 American deaths and over 450,000 recurrent attacks (Heron, 2008). Additionally, it is estimated that every minute an American will die from a coronary event and every 25 seconds someone will have a heart attack. Due to the relevance of CHD and myocardial infarction, there is a significant clinical need for vascular grafts in order to facilitate CABG procedures. As explained in the following sections, current sources of vascular grafts for CABG and other procedures are often not ideal or even adequate.

2.2 Physiology and Mechanics of Blood Vessels

Understanding the physiology and mechanics of blood vessels is a necessary step in developing graft replacements. Vascular tissue is composed of cells supported by extracellular matrix ECM. Blood vessels consist of three main layers. The outermost layer is the tunica adventitia, composed of a tough collagen fiber layer which provides tensile strength. The adventitia tends to be thicker in arteries than in the corresponding veins. The middle layer is the tunica media. This is primarily composed of smooth (involuntary) muscle cells (SMCs) and elastic fibers. Cells and fibers composing this layer are oriented circumferentially as seen in Fig 2.2.

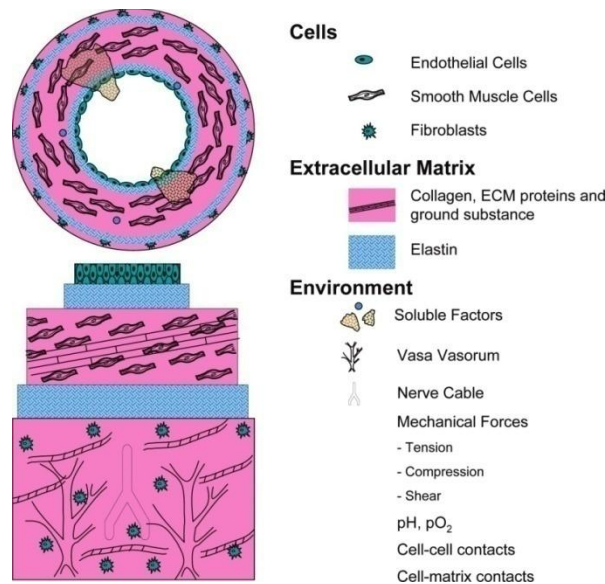


Figure 2.2: Structure of a Medium-Sized Blood Vessel

(Stegemann, 2007)

The tunica media is primarily responsible for physiological responses. This layer is much thicker in arteries due to the pulsatile nature of the blood pumping and higher pressures in these vessels. Since arteries receive blood from the heart, the pulses are stronger, while the pulsatile flow in veins has been diminished after passing through capillaries. Therefore, the arterial walls need to be stronger. The innermost layer is the tunica intima, composed of an endothelial monolayer separated from the tunica media by the sub-endothelial ECM layer. The intima provides crucial anti-thrombogenic properties in the lumen and is a selectively permeable barrier between the blood and the rest of the body. Elastic lamellae exist between both the adventitia and the media, and the media and the intima. The elastic lamellae thicknesses vary depending on vessel type and are primarily responsible for elastic recoil. It is likely that the intricate nature of native blood vessels need not be precisely replicated in order to generate clinically successful vascular grafts. Research has primarily focused on achieving vascular grafts with suitable compliance, biocompatibility, burst pressure, and anti-thrombogenic properties (Mitchell and Niklason, 2003).

2.3 Graft Replacement Sources

There are three main sources of grafts, autologous, synthetic and tissue engineered grafts. Although current technology is limited, solutions have been realized in each of these categories.

2.3.1 Autologous Grafts

In 2005 alone, 600,000 arterial bypass surgeries were performed with autologous grafts (Kielty et al., 2007). The two main sources of autografts for bypass are the Left Internal Mammary Artery and the Greater Saphenous Vein as seen in Fig. 2.3. The benefits of using autologous grafts are that there is no risk of rejection or immune response, and that they are completely natural vessels with functional non-thrombogenic endothelial monolayers. The use of these grafts involves additional surgery, although patients may lack suitable autologous vessels due to age, disease, or previous harvest (Schmelden et al., 2003). Veins used in place of arteries can also suffer compliance mismatch and insufficient mechanical strength. The additional risks and pain involved with the harvest surgery and the lack of suitable donor vessels in some patients creates a need for alternative graft sources in many situations.

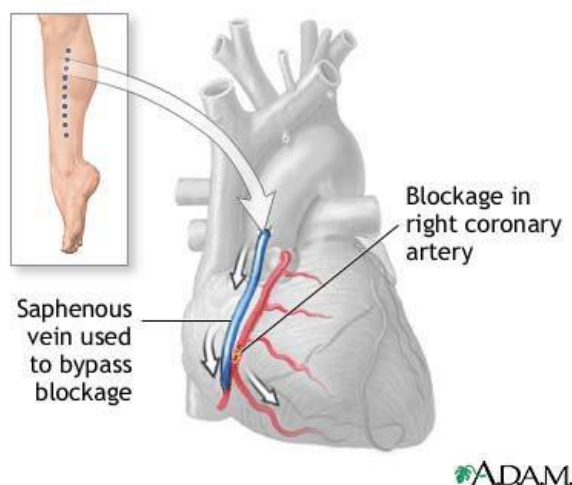


Figure 2.3: Autologous Graft Replacement

Found at: http://www.nlm.nih.gov/medlineplus/ency/presentations/100190_1.htm

2.3.2 Synthetic Grafts

When suitable autologous vessels are unavailable, completely synthetic grafts have been used, pictured in Fig. 2.4. Dacron and ePTFE are the main materials used in synthetic vascular grafts today (Ratner et al., 2004). In small diameter applications, these synthetic grafts are highly susceptible to clotting and failure due to a lack of a confluent non-thrombogenic endothelial monolayer. Upon implantation, the luminal surface of the synthetic graft is coated with plasma proteins, and eventually a platelet-fibrin aggregate (pseudointima). This is then covered with an endothelial layer, but only in a 10-15 mm zone from the anastomosis. The pseudointima that is not endothelialized can be subjected to SMC migration and proliferation leading to intimal hyperplasia, or trigger clot formation and thrombosis. Fibrous hyperplasia caused by an over active physiological repair response at the anastomotic site also often leads to failure of the grafts.



Figure 2.4: Synthetic Graft Replacement

Found at: http://www.atsmedical.com/uploadedImages/Public_Site/Products/Mechanical_Valves/BI1-1_avg.jpg

2.3.3 Tissue Engineered Grafts

The most common tissue engineering approach involves the use of exogenous scaffolds into which cells are seeded and cultured. These scaffolds provide structural support and allow for cell growth, migration, differentiation and cellular ECM production. Ideally, these scaffolds would degrade at the same rate as the natural tissue would proliferate and synthesize ECM. Tissue engineering scaffolds can be composed of

either natural or synthetic materials. An example of a tissue engineered graft is shown below in Fig. 2.5.



Figure 2.5: Lifeline Graft

Found at: <http://cytograft.com/technology.html>

2.3.3.1 Synthetic scaffolds

Synthetic scaffolds can be created from a variety of polymers such as polyesters, polyanhydrides, and polyphosphazenes (Yoon and Fischer, 2006). The initial chemicals and reactions necessary to synthesize these polymer scaffolds are incompatible with cell survival. Therefore the cells cannot be incorporated directly into the polymer scaffolds as they are formed. Instead cells must be seeded into the completed scaffolds posing difficulties in achieving uniform cell distribution and attachment. The scaffold degradation rate must be equal to the cellular proliferation rates and ECM synthesis in order to maintain graft structure and mechanical properties, and to avoid failure. Polyglycolic acid (PGA) and Polylactic co-glycolic acid (PLGA) are most commonly used as tissue engineering scaffold but are both resorbed rapidly (Isenberg et.al., 2006). It has been found that tailoring polymer scaffold degradation rates to correspond with varying cellular proliferation rates and ECM synthesis, while maintaining biocompatibility, poses a considerable challenge (Nerem and Ensley, 2004).

Although synthetic scaffolds provide initial mechanical strength and structure, compliance mismatch with native vessels has proven difficult to overcome (L'Heureux

et. al., 2006). This is due to the inability to match the mechanical properties of native vessels with synthetic vessels, which result in low compliance between the native tissue and graft. Thrombosis can result when mismatch between the mechanical properties of the native tissue and replacement occurs. Compliance mismatch quickly leads to the failure of implanted grafts due to the stresses of repetitive cyclic loading, which occurs when blood is continuously pumped through the vessel. The compliance of vascular grafts once the scaffold has resorbed is the result of cell generated ECM (Heydarkhan-Hagvall et. al., 2006). However, it has been shown that ECM production is inhibited in cells that are in contact with synthetic polymers (Nerem and Ensley, 2004). This finding has led many researchers to investigate alternatives to synthetic polymer scaffolds.

2.3.3.2 Natural Scaffolds

Natural protein scaffolds utilize the components of native ECM and create fully biological grafts. Natural protein scaffolds can be composed of collagen, elastin, fibronectin, or protein hydrogels (Stegemann et al, 2007). However, these scaffolds do not provide sufficient initial mechanical strength to support normal hemodynamic loading (Zhang et al, 2007). To improve mechanical properties and increase collagen fiber alignment, cross-linking has been increased and mechanical conditioning has been applied in vivo (Nerem and Ensley, 2004). Additionally, natural protein scaffolds are often difficult and expensive to manufacture (Stegemann et al, 2007).

Decellularized tissues are also used as scaffolds, composed of natural ECM, and as such offer improved biocompatibility. Animals can be used as a tissue source with the benefit of being readily available and inexpensive. The chemical processes through which the tissues are decellularized however, negatively affect the mechanical properties (Swartz et al, 2005). Chemical cross-linking is then required in order to attempt to restore suitable mechanical properties, which have yet to be tested in complex and risky situations such as a bypass-application (Stegemann et al, 2007).

2.3.3.3 Cell-Derived Vascular Grafts (No Exogenous Scaffolds)

Researchers are developing alternative approaches that avoid the use of exogenous scaffolds. By creating completely biological vascular grafts without the use of

exogenous scaffolds, it is likely that compliance match can be more easily achieved (Swartz et al, 2005). This approach requires that the cells synthesize their own ECM/scaffold. ECM production in cells can be stimulated through surface topography, chemical signaling, and mechanical conditioning (Swartz et al, 2005).

Cell sheets have been cultured and utilized to create layered vascular grafts. Monolayers of fibroblasts and SMCs have been cultured in medium enriched with ascorbic acid in order to stimulate production of collagen type I. The resulting vessels have high burst strengths of 2000 mmHg and perform well in suture pull out tests (L'Heureux et al, 2006). These collagen-rich cell sheets can be removed from the culturing surface without damage, which is essential. Multiple sheets are wrapped around a mandrel in order to form a tubular graft. Such grafts have recently been approved for use in clinical trials in blood vessel access applications (L'Heureux et al, 2006).

Cyto graft Tissue Engineering, in late 2008, reported results from their first clinical trial using sheet-based tissue engineered vessels (Konig et al., 2008). The multiply graft can be seeded with endothelial cells to produce a completely autologous tissue engineered blood vessel named LifelineTM. The cells for these clinical trials were sourced from patients with end-stage renal disease, lower-limb ischemia, or coronary artery disease, 25 patients in total. The engineered blood vessels were compared to native human vessels in patients with advanced cardiovascular disease in burst pressure, fatigue resistance with static or dynamic loading, suture retention strength, and compliance tests. This was the first demonstration of completely biological tissue engineered blood vessels implanted in humans (Konig et al, 2008). The vessels were implanted as either an arterio-venous shunt for dialysis purposes, or a lower-limb bypass, which have greater failure rates than upper-body replacements due to increased pressure. The results from this clinical trial provide benchmarks for future cardiovascular tissue engineering studies (Konig et al, 2008).

L'Heureux and his research team have been largely responsible for the success of vascular grafts generated through the utilization of cultured cell sheets. High patency and anti-thrombogenicity have been achieved in animal and human models with these grafts. Lengthy production times are required with these techniques however, and greatly hinder clinical implementation. Additionally, the over expression of collagen necessary to make

the grafts easy to handle, results in compliance mismatch and overly stiff grafts (Swartz et al, 2005).

2.4 Patents

An in-depth patent search was conducted to find completely tissue-engineered vascular grafts. Although there are similar patents using scaffolds and manipulated grafts, there is currently only one patent for a completely tissue-engineered vascular graft. Two patents and one patent application were found through the U.S. Patent and Trademark Office. The patents and applications were found to be the only ones that claimed to be able to produce completely scaffold free, cell derived tissue engineered grafts.

2.4.1 Patent # US 7,112,218 B2

Todd McAllister and Nicolas L'Heureux filed patent# US 7,112,218 B2 on September 26, 2006. This was for a bioreactor with mechanisms to grow tissue sheets and a rolling mandrel for the sheets to wrap around. This allows tissue sheets to be grown and then concentrically wrapped around a mandrel in order to create autologous blood vessels with little handling as seen in Fig. 2.6. There is a clamp for holding the sheet to the mandrel while wrapping the tissue. Fibroblasts are isolated from a skin biopsy, and cultured into a sheet. A supplementary rod with a control blade that rotates toward the sheet is used in order to separate 1-3 cm of the sheet's leading edge. This separated portion is then draped over the rollable mandrel. A magnetic clamp holds the tissue sheet to the mandrel, and remains in the roll to be taken out later. A Teflon coating over the mandrel prevents cell adhesion. Once the tubular vessel has matured, the cells are denatured. A cell sheet of fibroblasts is rolled over the denatured vessel. A suspension of endothelial cells is then placed in the lumen of the vessel to seed the lining. These vessels can be directly grafted into the patient or can be further conditioned mechanically with hemodynamic forces (McAllister et al, 2006).

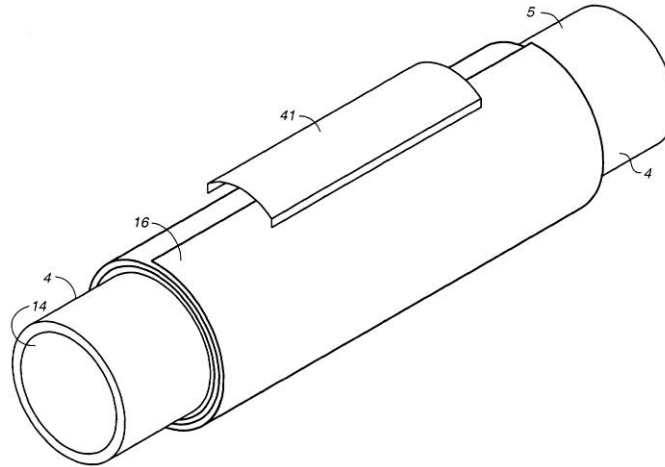


Figure 2.6 Rolling Mandrel (McAllister et al, 2006)

2.4.2 Patent # US 20,060,240,061 A1

Atala et al. of Wake Forest University developed a method utilizing a matrix seeded with endothelial cells, as stated in application US 20,060,240,061 A1. A biocompatible matrix is seeded with endothelial cells and there is a chamber that preconditions the matrix by moving biological fluid through the inner surface. This is a continuous flow that can be adjusted to control shear stresses in the vessel wall. Pulse rates and pressures are also adjusted to condition the tubes. The exterior can be preconditioned with this device by filling the preconditioning chamber with biological fluid. Therapeutic agents such as heparin can also be incorporated into the scaffolds. The scaffolds were created using electrospun fibers to allow greater flexibility and the creation of custom shapes. Teflon was also used in order to reduce cell adherence to the mold (Atala et al, 2006).

2.4.3 Patent Application # US 20,070,128,171

Another patent for tissue engineered blood vessels was completed by Tranquillo, Fig. 2.7, depicts the proposed blood vessel. Tranquillo developed a method to create engineered blood vessels which include an intimal layer surrounded by a smooth muscle media layer which are constructed around a tubular support. A matrix of endothelial cells

and smooth muscle cells form a bi-layer. This bi-layer forms a tubular structure around a support which is permeable to attractant factors. The application states that the formation of endothelial intimal layers should be surrounded by a smooth muscle medial layer, following the protocol that was conducted.

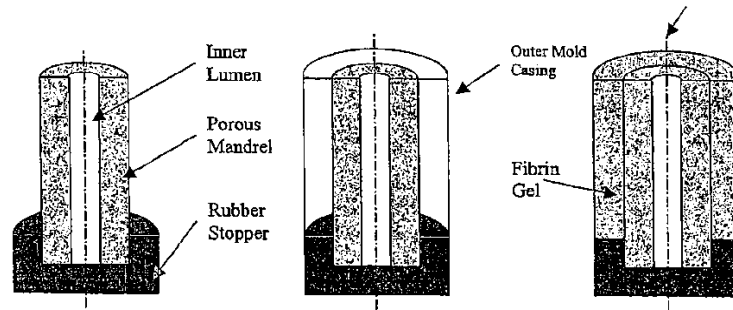


Figure 2.7: Drawing of Tranquillo's Layered Tissue Engineered Blood Vessel

2.5 Gaps in Current Technology

As previously discussed, current technology is lacking a suitable small diameter blood vessel replacement. The primary source for small diameter graft replacements is the autologous vessel, which requires a second surgery. The second surgery increases the risk of infection, pain from the operation and lengthens surgery time. Out of the 785,000 Americans who have a myocardial infarction, 470,000 are estimated to have recurrent attacks. These patients have a continued heart disease, as well as previously harvested vessels, making the resources for second surgeries limited (Lloyd-Jones et al, 2009). Coronary heart disease is estimated to cost \$165.4 billion annually through direct and indirect costs. Tissue engineered blood vessels for coronary bypass would greatly impact the state of the art and is only one possible application for these grafts. Implanting grafts as a dialysis fistula and replacing any vessel in the body, especially lower limb vessels, are other applications of a completely tissue engineered vessel.

The Cytograft technology is innovative and shows great promise for tissue engineering grafts. The task of completely cell-derived grafts is achieved, but the resulting grafts take about 6 months to produce, a very impractical timeline. Additionally, the clinical data was recently released, showing the results of several mechanical tests.

The *Lifeline* vessel replacement was compared to internal mammary arteries, and the results were drastically different in cases such as burst pressure and compliance. A native internal mammary artery has a burst pressure of 1599 ± 877 mmHg while the Cytograft vessel ranged from 3399-3523 mmHg, nearly triple. For compliance, the native vessel was 11.5%/100/mmHg while Cytograft was 3.4%/100mmHg, showing that the engineered tissue was much stiffer (Konig et al, 2008). While these vessels show promise and successful *in vivo* testing, there are still issues with mechanical properties that need to be addressed during culture.

Synthetic vessels work well in large diameter vessel replacements, such as aortic aneurysms, but can cause clots in small diameter applications. The immune response elicited from implanting a polymer and the lack of anti-thrombogenic endothelial cells make these ineffective in bypassing surgeries. The mechanical properties of synthetic grafts are also different enough to cause problems. Using tissue-engineered vessels can eliminate the immune response as well as eventually lead to closer mechanical properties to native tissue.

The bioreactor design itself is a great opportunity to address two large problems: mechanical properties by having cells self-assemble into the vessel geometry and cut down the growth time of tissue-engineered vessels. By using a cylindrical mandrel and a method having cells culture in the natural assembly of blood vessels, the current problems related to tissue engineered grafts may be eliminated. Cell alignment is crucial to the strength and properties of the vessels and the assembly process can influence the cell growth direction. Also, a bioreactor that facilitates self-assembly will reduce manipulation and culture time for layers to fuse after growing in sheets. The idea that cells can be put into a bioreactor and after a culture period, a blood vessel may be removed is the ultimate goal of this project. The approach of this project is to create a whole system that will enable sterile cell culture and growth of tissue engineered vessels, eliminating production time and potential for user error or concern.

3.0 Project Strategy

The design of a bioreactor to facilitate cell-derived vascular grafts was motivated by the need for small diameter vascular replacements. Throughout the design process the client's needs were assessed in order to direct the development of the project. In order to meet the client's expectations, interviews were conducted resulting in a client statement. From here, design objectives, functions, specifications, and constraints were established. Various design methods were utilized to further clarify the goals of the project. These methods included pairwise comparison charts, objective trees, and functions-means trees were used to guide the development of the design.

3.1 Initial Client Statement

Weekly interviews were conducted with our client and advisor, Dr. Marsha Rolle. In these meetings, the need for small diameter vascular replacements and the problems associated with current vascular graft designs were presented and discussed. The client's initial desires for the design were outlined in the initial client statement:

The goal of this project is to design a bioreactor to 1) generate totally biological vascular grafts by cellular self assembly into tubular structures (in the absence of exogenous scaffolds and cell sheets) and 2) support sterile culture and conditioning of the resulting vascular grafts. The ultimate goal of the project is to create a device that will reproducibly generate tissue engineered blood vessels with physiological and mechanical properties suitable for transplantation.

The above client statement was expanded and revised as interviews continued and as sufficient background research was conducted.

3.2 Objectives & Constraints

Key goals of the project were determined from the revised client statement and expanded into a complete list of objectives and constraints. The list was split into objectives for the bioreactor design and objectives for the vascular grafts. Pairwise comparison charts (located in Appendix A) were used to weigh the importance of each objective and constraint. The objectives were broken further into sub-objectives and both were compiled in an objectives tree shown in Fig. 3.1 and 3.2.

3.2.1 Bioreactor

After an in-depth discussion on what features, attributes and functions the bioreactor should satisfy, objectives and constraints for the bioreactor were determined. In order to fulfill the client statement, the bioreactor was designed to produce tissue in a cylindrical form so that no tissue manipulation is required to produce a graft. Not only should this bioreactor produce grafts quickly, it should also be easy to use, safe and provide a continuous nutrient delivery. Finally, the bioreactor should be able to produce more than one tissue tube at a time and should be inexpensive to produce and grow the grafts. Objectives of the bioreactor are shown in Fig. 3.1.

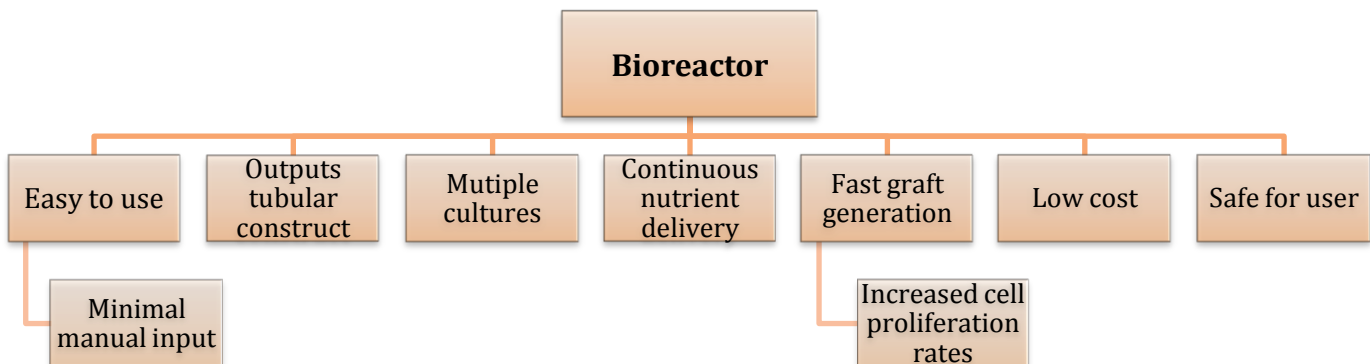


Figure 3.1: Bioreactor Objectives

There were also numerous constraints that needed to be taken into consideration when designing the bioreactor. Time and budget were driving constraints; the project needed to be completed with a budget of \$624.00 and by April 30th, 2009. The facility used to create the bioreactor was also a constraint because the project was completed in

an assigned lab area in Salisbury Laboratories at Worcester Polytechnic Institute. Space was limited and the bioreactor needed to fit within the allotted space in the incubator.

3.2.2 Vascular Grafts

In order to design and build a bioreactor, it was also important to keep in mind the desired objectives for the vascular grafts that would be produced by the bioreactor. The bioreactor should be able to produce vascular grafts in a manner that is reproducible, so that not only each graft is the same, but also so that the bioreactor produces the same cell derived graft each time it is used. The design should also incorporate a way to influence the grafts to self generate Extracellular Matrix (ECM). When the bioreactor has produced vascular grafts, these grafts should also be suturable, easy to handle and should be able to withstand the pressures of a natural tissue which is around 1599 ± 877 mmHg (Konig et al, 2008).

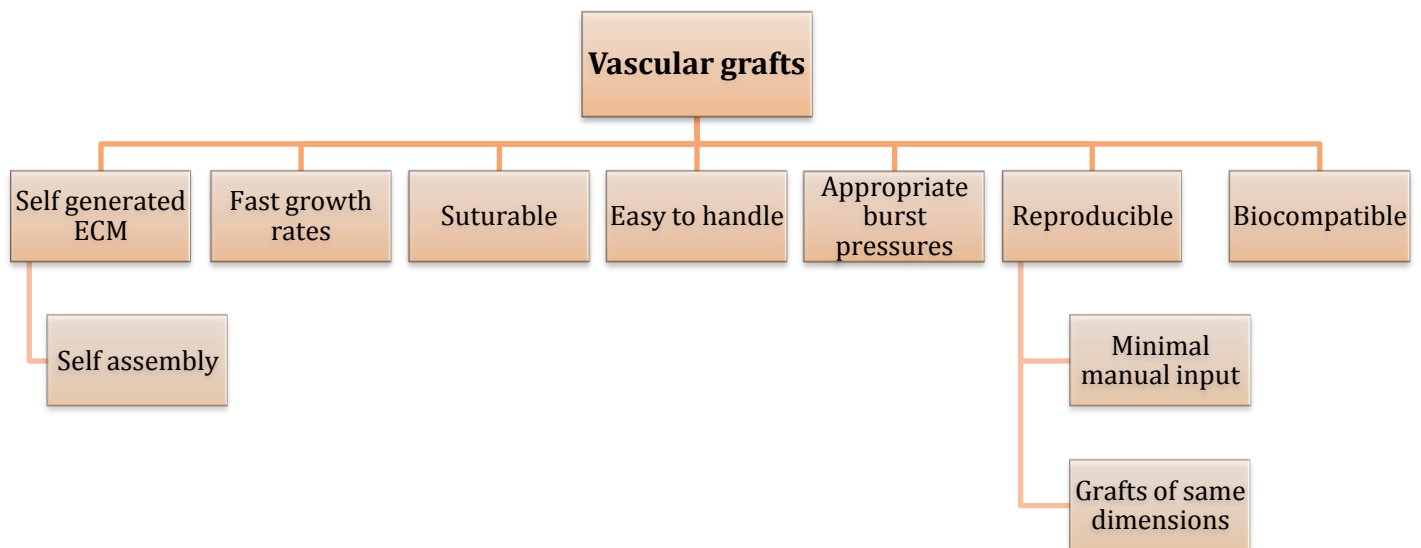


Figure 3.2: Vascular Graft Objectives

3.3 Revised Client Statement

A revised client statement was developed through determining the objectives and constraints of the project. The revised client statement is as follows:

The goal of this project is to develop a method to quickly generate multiple vascular grafts through the design of a novel bioreactor. The method and design should stimulate self assembly of cells introduced to the bioreactor, without the use of exogenous scaffolds. The design should produce reproducible tubular grafts that have self generated extracellular matrix. Additionally, the necessity for graft manipulation should be minimized so that the vessels are produced quickly and easily. The completely biological vascular grafts should be approximately 2 mm in diameter, 5 cm in length, and 250 μm in wall thickness. The resulting grafts should be suitable for transplantation: sutureable, easy for surgeons to handle, strong enough to withstand pulsatile flow and biocompatible with the recipient.

3.4 Needs Analysis

In order to determine which objectives were most important, a pairwise comparison was used and can be found in Appendix A. Scores were given based on conversations with the client and calculated appropriately. Needs and wants of the design were narrowed down based on scoring. This weighted objective tree for the objectives of the bioreactor is shown in Fig. 3.3. The safety objective was eliminated from the tree as it is always considered throughout the process and was deemed the most important by the client. Through additional conversations with the client it was determined that developing and validating the bioreactor design was the main objective of the project, which was continued throughout the remaining portion of the project.

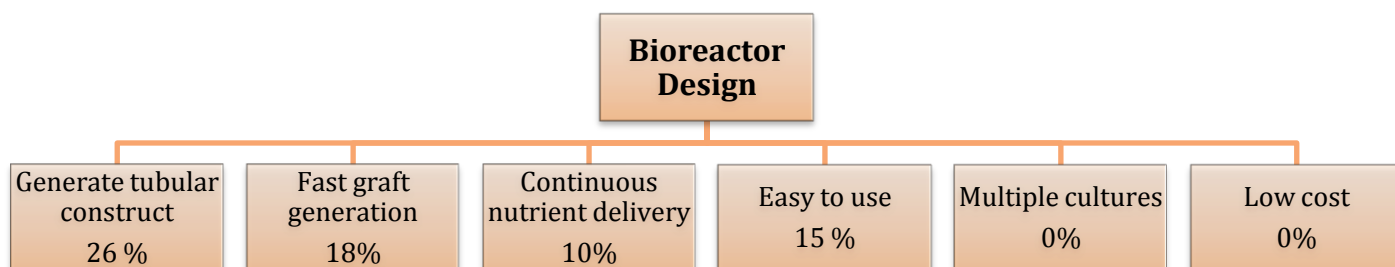


Figure 3.3: Weighted Objective Tree based on needs analysis

3.5 Bioreactor Functions

In order to meet the objectives of the bioreactor, desired functions of the bioreactor were split into three main categories. The categories and the functions of the bioreactor are listed in Table 3.1. The design of the bioreactor consisted of determining the best structure to assemble the cells, the method to assemble and culture cells and the housing to culture and monitor tissue.

Table 3.1: Bioreactor Functions

Functions of Bioreactor	
Structure to Assemble the Cells	
	Creates/Supports tubular construct
	Allow removal of intact tubular construct
Method to Assemble and Culture Cells	
	Facilitate cell seeding
	Promote cellular growth
	Promote cell adhesion
Housing to Culture and Monitor Tissue	
	Allow nutrient diffusion/ gas exchange
	Allow for visualization of fibers
	Housing for fibers
	Fixation of Fibers
	Vacuum adheres cells to mandrel
	Closed off housing
	Fibers are easily removed

3.6 Project Approach

Once the functions of the bioreactor were clearly established, the design of the bioreactor began in three distinct categories to be designed and verified. These three design categories were examined and analyzed in parallel to determine the optimal means to create the bioreactor as seen in the morphological chart in Appendix B and design matrixes in Appendix C. As pictured in Fig. 3.4, the bioreactor functions were split up into three main categories which were; structure to assemble cells, method to assemble and culture cells and a housing to culture and monitor tissue. Each of these three categories were investigated in Chapter 4 and later verified in Chapter 5.

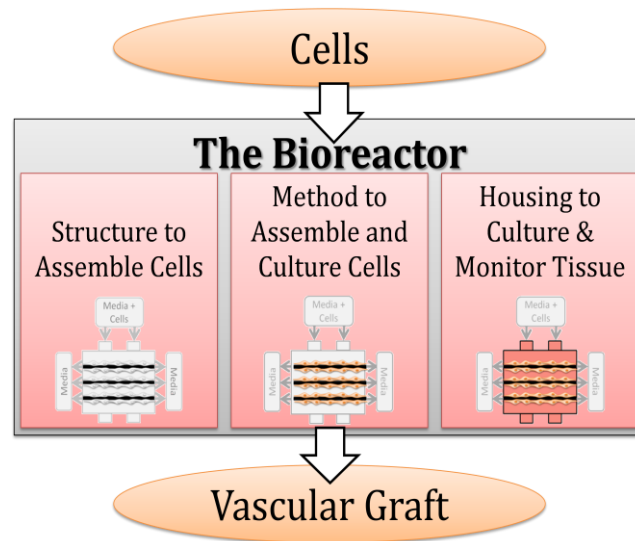


Figure 3.4: Three Function Groups of Bioreactor

4.0 Alternative Designs

Once the project objectives were weighted by importance in designing the bioreactor, conceptual designs were generated. Through initial brainstorming, functions were developed and it was decided that cells would need a surface to adhere to in order to meet confluence and create a tubular structure. With the functions in mind, different designs were investigated to meet the functions and objectives of the bioreactor. Additional components were added to a traditional bioreactor to promote cell growth and cell seeding. A custom cartridge in which to culture the vascular grafts would be necessary. The compiled functions helped in creating an overall schematic of how the bioreactor would be assembled, which is shown in Fig. 4.1.

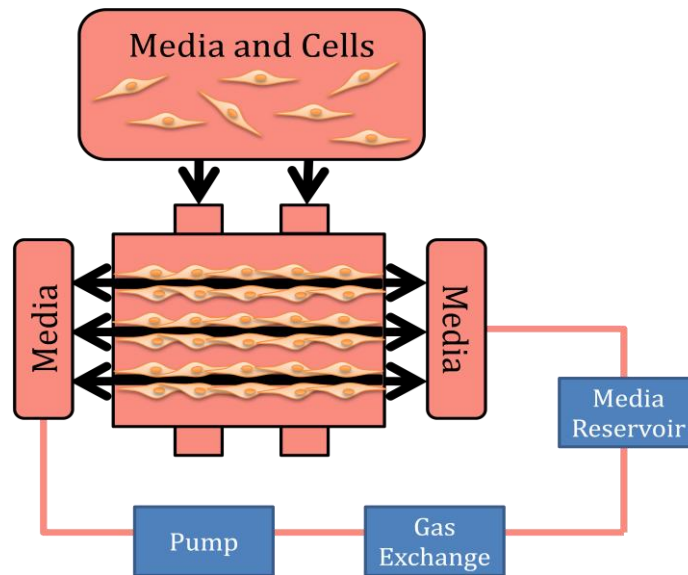


Figure 4.1: Bioreactor Schematic

The conceptual designs were chosen with the functions in mind. Controlling the cells as they grow eliminated the step of manually rolling sheets of tissues into tubes, which is currently a critical step in tissue engineered grafts

4.1 Structure to Assemble Cells

The first function category that was investigated was the structure to assemble the cells on, the schematic depicted in Fig. 4.2. The options were split up into a group of

cylindrical mandrels upon which to culture the cells a mechanical mandrel idea, which would allow for flat cell growth and a porous mandrel idea which would allow for nutrient diffusion.

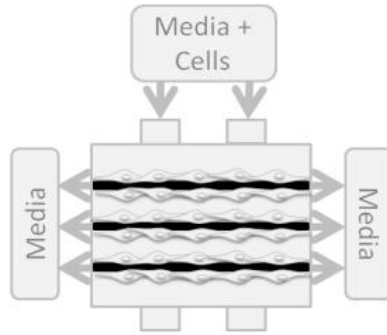


Figure 4.2: Structure to Assemble Cells

4.1.1 Mandrel Designs

In order to culture cells directly into tubular structures, four main design categories were developed. These four design categories all involved the use of tubular forms or mandrels upon which the cells would be directly cultured and self assembled into tubular vessels. The first design category utilizes a cylindrical mandrel the size of the inside diameter of the required vessel and can be seen in Fig. 4.3. The cells could be cultured into a vascular graft around the outer surface of the mandrel.

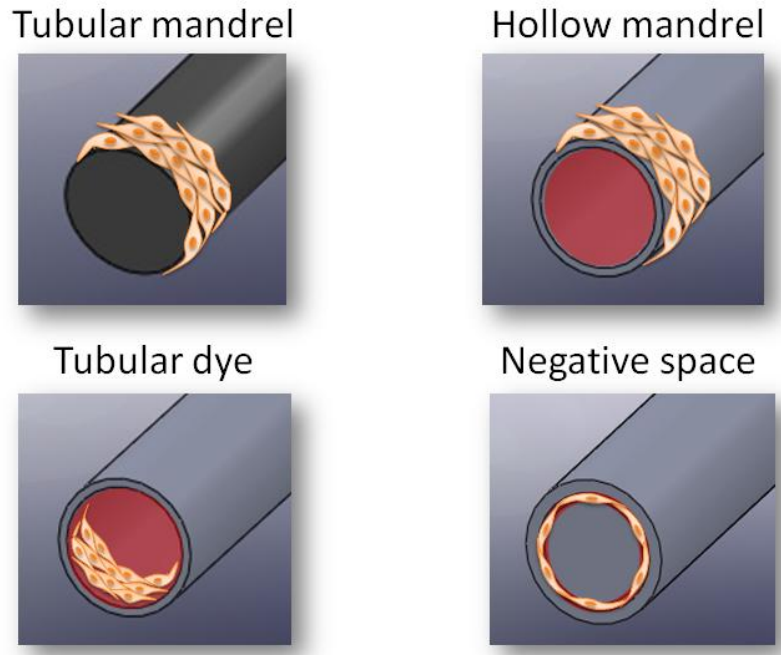


Figure 4.3: Mandrel Designs

The second category would utilize a tubular dye. Cells could be cultured on the inner surface of the dye, as seen in the Fig. 4.3. The dye would have an inside diameter equal in size to the outside diameter of the required vascular graft.

The third design, as pictured in Fig. 4.3, would allow for cell culture around an outer diameter, equal in size to the desired inner diameter of the vascular graft. One option for a hollow mandrel that was investigated in depth was hollow fibers, which are used in filtration cartridges and hollow fiber bioreactors. Hollow fiber bioreactors, as seen in Fig. 4.4, create an artificial capillary system in a cartridge so that higher cell densities and proliferation rates can be achieved as compared to standard flask or plate culture techniques. Fibercell Systems Inc. produces a hollow fiber bioreactor with fibers that are 1.3 mm in outside diameter.



Figure 4.4: FiberCell Cartridge

Media circulating through the porous hollow fibers delivers glucose, oxygen, and nutrients to the cells, and at the same time carries metabolic waste products as seen in Fig. 4.5. Media flow through the porous hollow fibers also ensures that cells attached to the mandrel receive the same nutrient diffusion from inside and outside the fibers as the cells interacting with the media inside the main cartridge.

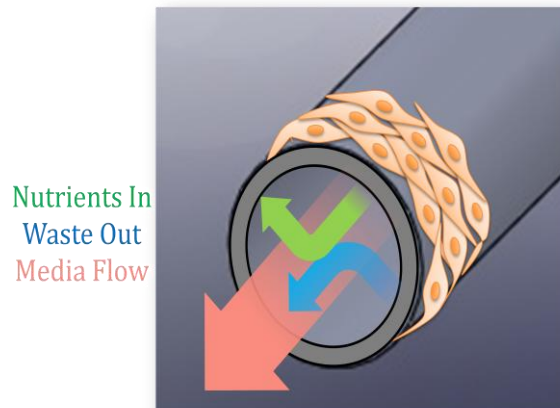


Figure 4.5: Oxygen, Nutrient and Waste Flow through Fibers

The fourth and final design category, as seen in Fig. 4.3, would utilize a mold in which the vascular graft would be cultured. The negative space created by the mold would be the size of the required vascular graft. This mold would also control the wall thickness of the graft by establishing both the inside and outside diameters. The negative space mold approach constrains the cells within a space such that the cells needn't necessarily attach to the surface. This design was similar to a conventional negative space mold. The challenge in this design was to keep the cells dispersed throughout the mold, as the force of gravity would naturally draw them all to the bottom. By rotating and or vibrating the mold in various directions it could be possible to keep a media and cell solution evenly distributed throughout the mold until the cells proliferate sufficiently to form a unified tubular construct.

These approaches require some method of maintaining the cells on the surface of the die or mandrel in opposition to the force of gravity. Until the cells attach sufficiently to the surface, or confluence is reached and cell to cell attachment is sufficient, gravity will prevent cells from maintaining contact with the surface of the die or mandrel. This problem would not be encountered in cell sheet culture as gravity keeps the cells arranged on the flat surface upon which the sheets are cultured.

4.1.2 Mechanical Mandrel

As an alternative to using a mandrel that is initially tubular, the possibility of using a structure that was initially flat was also considered. This would allow the cells to be easily seeded and cultured into sheets, since cells are commonly grown on a flat dish. Once the cells reached confluence or sufficient cellular attachment to the structures surface was achieved, the structure could roll up into either a tubular die or mandrel. A close view of the flat roll-able mandrel can be seen in Fig. 4.6.

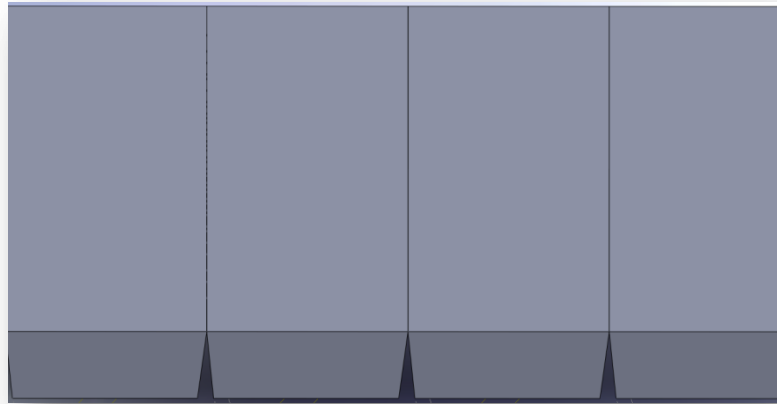


Figure 4.6: Flat Mandrel before Rolling

The cells could then be further cultured into the final tubular construct, as seen in Fig. 4.7, by allowing the cells to proliferate until the seam between the two ends of the sheet were fused and the appropriate wall thickness was achieved. These mechanical mandrels or dies would be made of a flexible culture surface or membrane, attached to a mechanical, segmented structure.

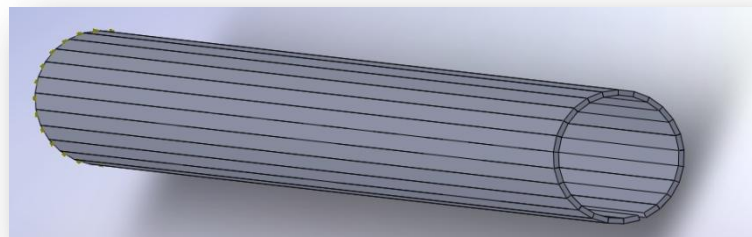


Figure 4.7: Mechanical Mandrel Rolled

The mechanical mandrels or dies investigated fell into two categories. The first involved the use of multi-component mechanical assemblies that could easily convert from flat to tubular structures. These mechanical dies or mandrels would have to create inside or outside graft diameters of only a few millimeters. The second category of mechanical die or mandrel involved the use a shape memory alloy such as nitinol. This could eliminate the need for complex multi component structures, since these shape memory alloys can convert from one shape to another based on temperature changes or

other external stimuli. A flat membrane could be lined with simple nitinol bands that could alternate between flat bands and round hoops, thus creating a construct that transforms from a flat into a tubular structure when the appropriate external stimulus was applied.

4.2 Method to Assemble and Culture Cells

The second design function that was investigated was the method to assemble and culture the cells on. The selection of the method was also determined by the structure to assemble the cells. Four methods to seed the cells onto a mandrel were investigated. Static seeding, a method to manually rotate the cartridge, and an automated rotation system and a pump/vacuum system led to the final decision, which was determined through design verification. This design function is highlighted from the overall schematic in Fig. 4.8.

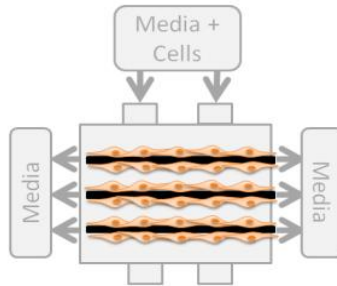


Figure 4.8: Method to Assemble and Culture Cells

4.2.1 Static Seeding

The simplest seeding method would be a static seeding method in which the cells would be seeded into the chosen housing of the bioreactor. In the case that a mechanical mandrel was used, static seeding would be an option. Static seeding is a common method in which cells are simply placed in a flask and allowed to grow into cell sheets.

4.2.2 Manual Rotation

Avoiding the use of cell sheets and mechanical mandrels would be achieved by allowing the cells to attach in series to a slowly rotating mandrel or dye surface. Sufficient attachment strength would need to be achieved before the surface was rotated such that gravity opposes the cell to surface attachment. This would have required a very slow rate of rotation and perhaps an equally slow introduction of cell suspension to prevent cell aggregates from forming instead of cell to surface binding.

4.2.3 Automated Rotation System

An automated rotation system would have eliminated the need to manual rotate the cells and could be controlled. This would have been able to eliminate the uncertainty involved in manually rotating cells for a specified time and would also eliminate the manual work required, better meeting the objectives of the bioreactor.

4.2.4 Pump/Vacuum System

Another possible method for maintaining the cells on the surface of a mandrel, involved the creation of a vacuum at the surface resulting from an inward flow of media. This flow would maintain the cells on the surface until they attach to the surface or proliferate sufficiently to aggregate and provide their own mechanical support, Fig. 4.9. The use of a porous construct would be required so that media could be drawn through the surface to create a vacuum. Media flow could also provide nutrient and gas exchange and thus provide biological support as well as mechanical support.

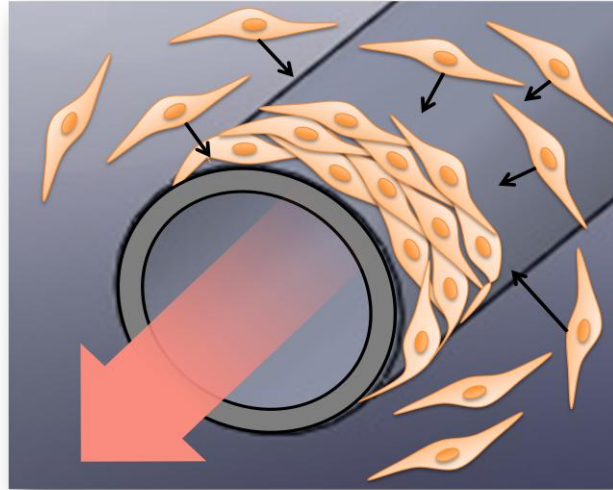


Figure 4.9: Cell Attachment to Surface

4.3 Housing to Culture and Monitor Tissue

The third function of the bioreactor was an essential step in developing bringing each of the components together. It was necessary to take into account each of the objectives, constraints and functions in order to produce this central piece of the system. Design alternatives for the cartridge were generated using a morphological chart located in Appendix B and qualified using a decision matrix found in Appendix C. The housing to culture and monitor tissue, Fig 4.10, was designed to allow constructs to be easily removed and installed, cells to be seeded directly into the cartridge and to accommodate multiple cultures. Additionally the design was expanded to allow for housing, fixation, and visualization of the cultures and facilitation of cell dispersion during seeding. Additionally the design of the cartridge should incorporate a material that should inhibit cell adherence.

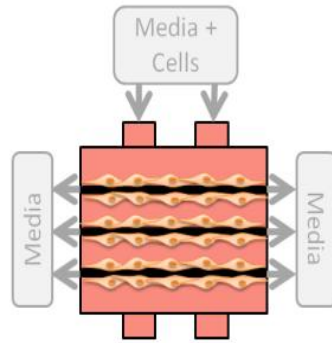


Figure 4.10: Housing to Culture and Monitor Tissue

4.3.1 Tubular Construct Orientation

The decision matrices found in Appendix B and C focused on the shape of the cartridge. Cylindrical and flat methods to orientate the tissue constructs were considered. The cylindrical design would place the constructs around the perimeter of the shape while in the flat design fibers would be fixed in a straight line as seen in Fig.4.11.

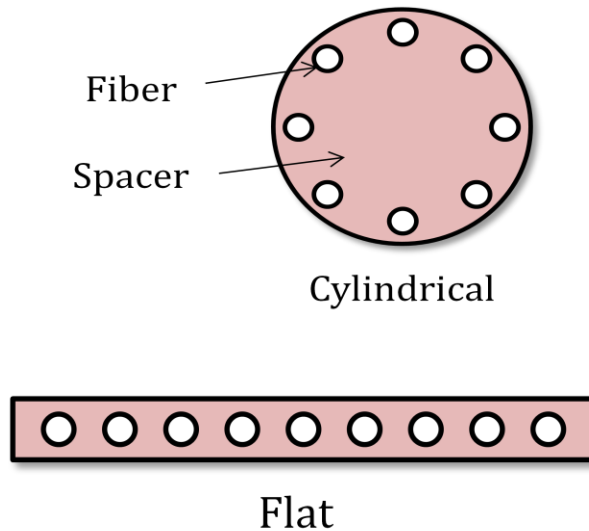


Figure 4.11: Cartridge Shapes

4.3.2 Cartridge Designs

SolidWorks 2008 was used to make detailed drawings and 3-D representations of novel cartridge designs. The spacer and end cap together are referred to as a manifold. The housing device was attached to the rest of the assembly by screws which brought the

four pieces together to form the cartridge housing. The pieces were designed so that they could be screwed together, allowing for access to the constructs. The cartridge design shown in Fig. 4.12 incorporates the cylindrical formation of the grafts where the cartridge design in Fig. 4.13 incorporates the flat orientation of constructs.

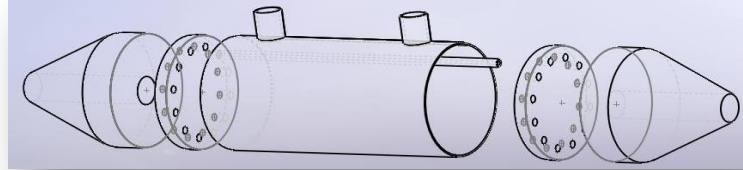


Figure 4.12: Cylindrical Cartridge Design

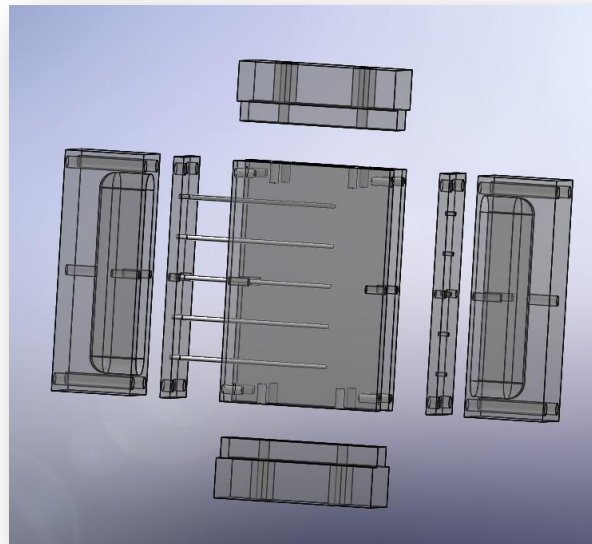


Figure 4.13: Flat Cartridge Design

5.0 Design Verification

In order to verify the design of the bioreactor, the three main functions were tested in parallel. The final design aspects that were chosen were the porous hollow fibers to be used as the support structure to grow the cells on. The vacuum system was chosen as the seeding method and the flat cartridge was chosen as the housing to monitor and culture the tissue. These aspects were chosen based on the decision matrices in Appendix C and conversations with the client. The hollow fiber mandrels allowed for vacuum seeding and artificial cell culture, satisfying two functions of the bioreactor. Therefore, the housing design was based off the size of the fibers as seen in Fig. 4.13. and 5.1.

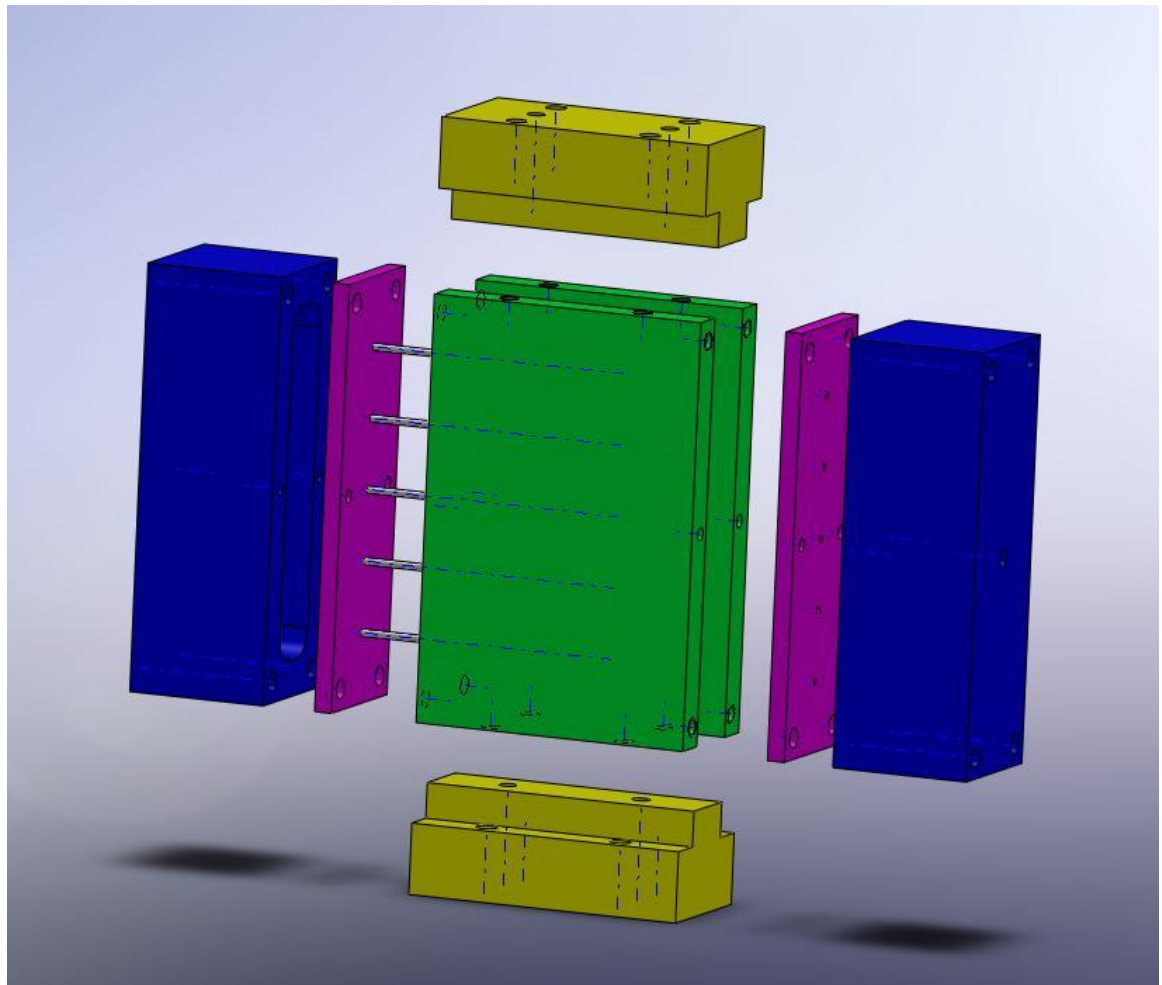


Figure 5.1: SolidWorks Cartridge

The support structure to assemble the cells was tested by verifying that the porous hollow fibers (polysulfone+ FiberCell) could support cell growth and to determine a

method to visualize cell growth without removal from the bioreactor's cartridge. The cell assembly and culture methods were also tested and analyzed using Fluent computational fluid dynamics software and pump testing. The housing to culture and monitor the tissue was also tested using Fluent and additional visualization testing.

5.1 Verification of Support Structure

Assuming the bioreactor would be used for research purposes, growth within the bioreactor needed to be assessed without compromising the tissue. When observing the fibers using a compound microscope the fibers appear black. Therefore it was essential to develop a method to distinguish the polysulfone+ fibers, shown in Fig 5.2, from the HDF cells in order to assess cell attachment and growth. Additionally, the compatibility of polysulfone+ fibers with cell growth was observed. The two methods used to verify the support structures, the hanging drop and the v-well, which are explained in more detail in Section 5.1.1 and 5.1.2.

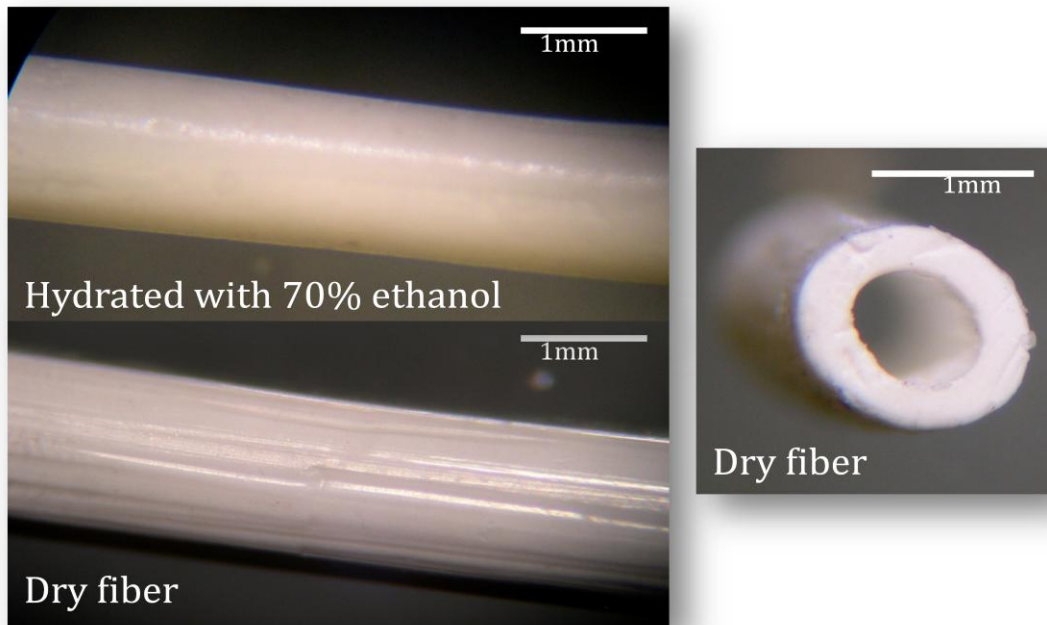


Figure 5.2: Magnified Fibers (Hydrated and Dehydrated)

Human Dermal Fibroblasts (HDFs) were cultured starting at passage 12. Cells were observed approximately every 24hrs, fed approximately every 48 hrs, and passaged when observed to be 80-100% confluent. Cells were cultured using DMEM which contained approximately 10% Fetal Bovine Serum (FBS) and approximately 1% Pen-Strep. Before utilization in experiments, the number of viable cells was measured using Trypan Blue in order to make the specified cell concentration for experimentation. Trypan Blue stains dead or dying cells while viable cells are not dyed. A 1:1 solution of Trypan Blue and cell solution (40 μ L each) was made and micropipetted into a hemocytometer used to count the cells.

In order to incorporate the polysulfone+ fibers in tests and the bioreactor design they first needed to be removed from the FiberCell cartridge which contained 20, 10cm long fibers. This process was carried out in a sterile environment and with sterile tools in order to ensure that the fibers would not be contaminated. All materials used to open the cartridge were sterilized using an autoclave for 15 minutes at 121°C and all procedures were performed in a fume hood, Fig. 5.3.



Figure 5.3: Materials Used to Open FiberCell Cartridge

A disposable sterilized towel was placed on the work surface of the fume hood to decrease the risk of contamination. A saw was used to remove the end-caps of the FiberCell cartridge and sterile surgical scissors were used to separate the polysulfone+

fibers which were embedded in polyurethane. Forceps were used to place the fibers on the sterilized towel so that they could be cut into the specified lengths. The fibers were cut using a razor into ten 6cm fibers and ten 4cm fibers. The 4cm fibers were then cut in half resulting in twenty 2cm fibers. The fibers were placed using forceps in 15mL sterile centrifuge tubes in order to store them without risk of contamination. The fibers were separated into six different centrifuge tubes so that there were 5 fibers in each tube in order to further prevent contamination.

It was necessary to precondition the polysulfone+ fibers before they could be successfully used in cell culture. The preconditioning was in accordance with the protocols outlined in the FiberCell guide (Hollow Fiber Bioreactor User's Manual, 2008)

Only the 2cm fibers were statically preconditioned while the 6 cm fibers were kept for use in the completed bioreactor design. The fibers were soaked in ethanol, phosphate buffered saline (PBS), cell culture media according to the procedures outlined in Sections 5.1.1 and 5.1.2.

5.1.1 Verification Using Hanging Drop Method

The hanging drop method is a technique utilized to promote cell adhesion to a tubular construct using the forces of gravity. This technique was used to determine the methods that would allow the best visualization of HDF attachment, confluence, proliferation, and viability on the polysulphone+ mandrel. Polydimethylsiloxane (PDMS) was mixed with 10% curing agent and poured into a cylindrical mold. A small piece of silicone tubing (about 1.3mm OD and 2cm length) was placed on the PDMS to form a groove where the polysulphone+ fibers would sit. The PDMS filled mold was placed in an oven at 80°C for 45 minutes. The cured PDMS rings were removed from the mold and sterilized using an autoclave. A 2cm polysulphone+ fiber was placed in the groove and fixed using sterile silicon glue.

The glue was allowed to set for 30 minutes. The PDMS rings, with attached fibers facing up, were fixed using silicon glue in the middle of three six well plates, one per well until a total of 16 wells contained the PDMS ring and attached fiber. This set-up is shown in Fig. 5.4.

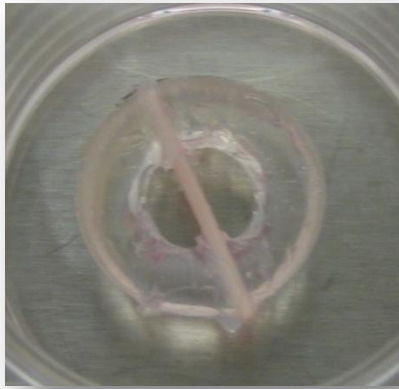


Figure 5.4: PDMS Ring Attached to Fiber

Two sets of hanging drop experiments were carried out. In the first set of experiments, the polysulfone+ fibers were preconditioned by soaking in ethanol for 15 minutes and soaking in cell culture media for approximately 72 hours after the six well plates were prepared for the hanging drop experiments as outlined above. CellTracker, a florescent dye, was used to label the cells to determine if the cells attached to the fibers. CellTracker was added to suspended cells and 3 of the 16 wells were seeded with cells loaded with CellTracker. Next 14 wells were seeded with a cell solution that did not contain CellTracker. The remaining well was used as a control and left unseeded. A micropipet was utilized to place a cell solution of 280 μ L at a concentration of 10^6 cells/mL into the center of each PDMS ring except the control which received 280 μ L of blank cell culture media. Remaining cell solutions were plated in a 75cm³ cell culture flask. The plates were flipped and the surface tension allowed the drops of solution to hang without falling so that they surrounded the fibers as shown in Fig. 5.4.

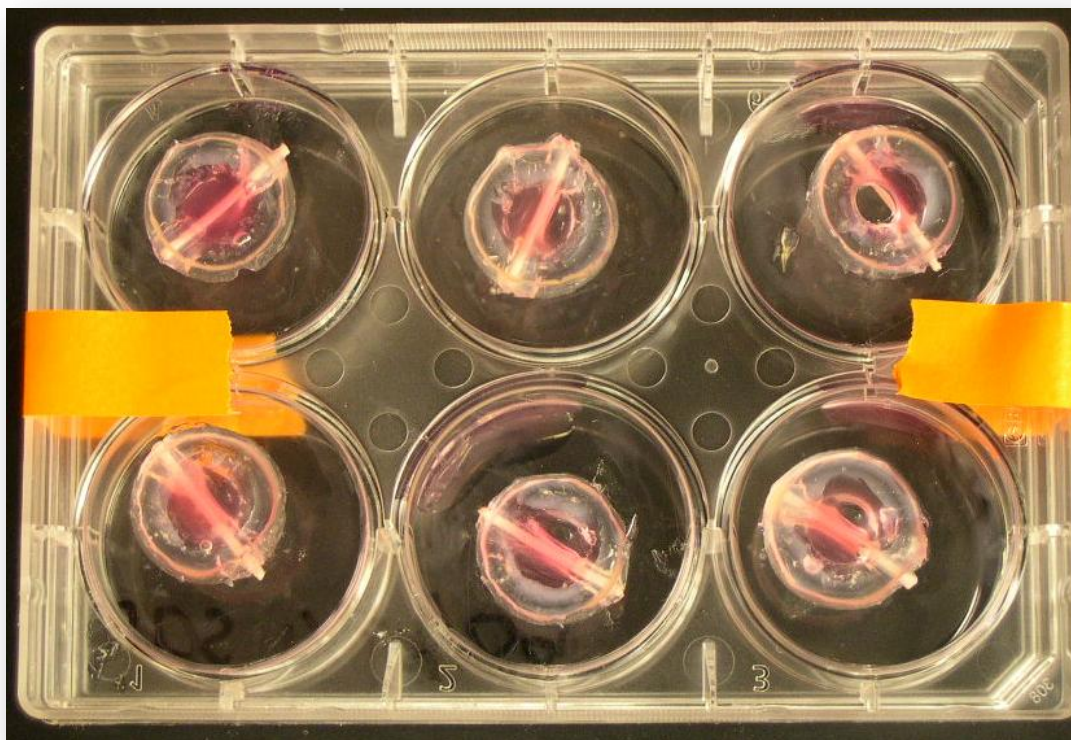


Figure 5.5: Hanging Drop Plates

At this point, the plates were allowed to incubate for 1hr. After cell attachment the plates were flipped back to their normal orientation. The remaining solution was aspirated and 6mL of fresh media was added to each well as shown in Fig. 5.6.



Figure 5.6: Media in a Hanging Drop Well

The wells that contained cells with CellTracker solution were viewed using a florescent microscope after the 1 hr seeding period. The observed fibers were fluorescing but the control also fluoresced. The plated cells that contained CellTracker were viewed using the florescent microscope and the cells fluoresced much brighter than the fibers. At this point it was determined that cells had not attached onto any of the fibers that were seeded with the CellTracker cell solution. After 3 days CellTracker solution was added to 3 wells seeded normally (not loaded with CellTracker). Once again no observable difference could be determined when the seeded wells were compared to the controls. Once again it was determined that no cells had attached to these fibers. Although the protocol developed for this experiment outlined instructions to add CellTracker to 3 wells every 3 days, the experiment was stopped at this point since no cell attached was observed.

The cells plated with CellTracker were observed over a 48 hour period to determine the effects of CellTracker on cell viability. It was observed that the cells loaded with CellTracker were not as healthy as unmodified cells. From these observations it was determined that loading the cells with CellTracker before cell seeding could be affecting cell attachment. The protocol for the second hanging drop experiment was modified based on the results of the first experiment. Furthermore, on second examination of the FiberCell Bioreactor guide, it was determined that in order for cell

attachment to occur on the polysulfone+ fiber, they must also be preconditioned with PBS.

A second hanging drop experiment was therefore set up with an adapted protocol. For this experiment the fibers were preconditioned by soaking in ethanol for 15 minutes, soaking in PBS twice for 5 minutes each, and finally soaking in cell culture media for approximately 72hrs. For this experiment the cell culture media was changed every 24hrs during that portion of the preconditioning. No cells were seeded with CellTracker however CellTracker was planned to be added to 3 wells, 3 days after seeding the fiber. One day after cell seeding, fibers were viewed using a stereomicroscope and compared to the control which had no cells added to determine if cells had attached to the fibers. At this point the experiment looked promising but it could not be definitively determined that cells had attached to the fiber. Two days after cell seeding the plates were observed and certain wells were found to be contaminated. Therefore the plates were bleached and disposed of.

At this point the team determined that a method with fewer variables and less risk of contamination should be implemented in order to verify the support structure chosen for the bioreactor design. It was determined that the hanging drop method could not generate reproducible results since it relied heavily on user manipulation. This extensive manipulation also increased the risk of contamination. Fig. 5.7 shows the inconsistencies of the hanging drop method.

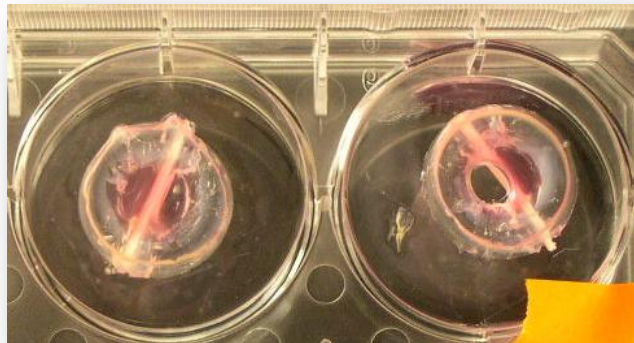


Figure 5.7: Inconsistency of Method

As can be seen in Fig. 5.6 the well on the right did not result in a uniform hanging drop. Wells which resulted in insufficient hanging drops were marked so that these inconsistencies could be considered when analyzing the results.

5.1.2 Verification Using the V-Well method

Since the hanging drop experiments were both unsuccessful, a different seeding method was necessary in order to verify the chosen support structures. Therefore, the V-well method, developed by Shawn Carey, Jon Charest, Jason Hu, and Elizabeth Ellis (Carey et al, 2009) was carried out. A machined mold, developed by Carey et.al, to allow cell attachment around the entire diameter of a tubular construct was placed in a 100mm cell culture plate and PDMS with 10% curing agent was poured into the mold. The PDMS was cured by placing it in an oven set at 80°C for 1.5hrs.

The polysulfone+ fibers used for this experiment were preconditioned in a 15mL centrifuge tube. First the fibers were soaked in 8mL of ethanol for 15 minutes. Next the fibers were soaked in PBS twice for 5 minutes each. Finally the fibers were soaked in cell culture media for approximately 120hrs changing the media every 24hrs. Two 2cm fibers were placed in each of the three wells. Two of the wells were seeded with a cell concentration of 1.85×10^6 cells/mL. The remaining well was left unseeded to be used as a control. The fibers were left in the cell solution for 1.5hrs at which point the solution was aspirated and replaced with fresh media as shown in Fig. 5.8.

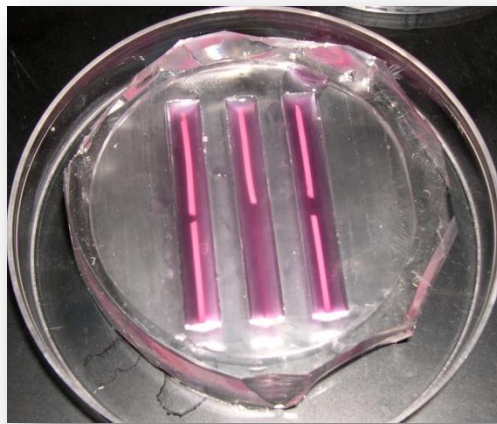


Figure 5.8: V-Well Method

After three days, a 5 μ M concentration of CellTracker was added to each well. The cells were then observed using a fluorescence microscope. As observed during the hanging drop experiments, it was difficult to differentiate the fluorescence of the cells from that of the fiber. Additionally, it was difficult to observe the fiber while in the V-wells and therefore the fibers were sacrificed from further experimentation by removing the cover of the plate and placing one seeded fiber and one control fiber in a 5mm cell culture flask filled with 6mL of cell culture media. Once again the fibers were observed using the fluorescence microscope and compared. Fig. 5.9 shows the unseeded (left) and seeded (right) fibers.

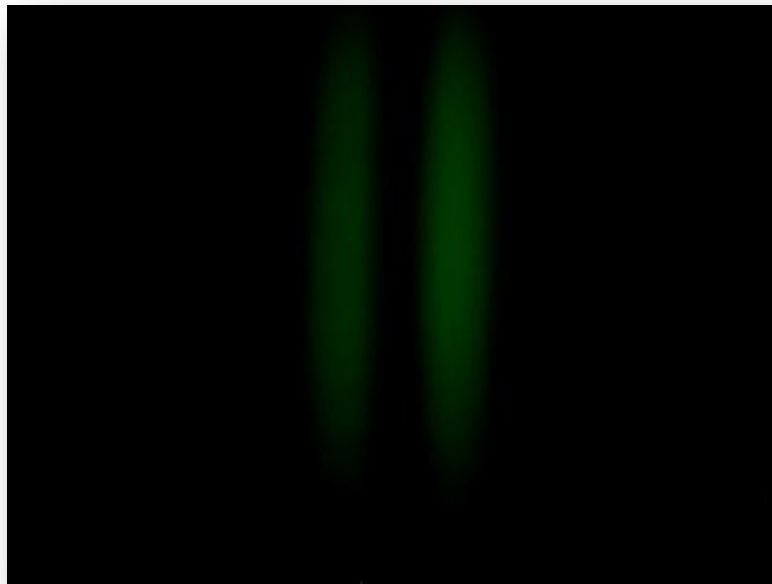


Figure 5.9: Unseeded and Seeded Cell Tracker Fibers

Although the seeded fiber (right) seems slightly brighter and thicker than the unseeded fiber (left), this observation cannot be definitively quantified. The media in the wells was then aspirated and the fibers were trypsinized and Trypan Blue dye was used in conjunction with a hemocytometer in order to determine a cell count if any. From the cell count it was determined that there was an average of 40,000 cells per fiber confirming that cells had in fact attached to the fibers. From these results it was determined that a fluorescent dye would not be sufficient means of visualizing cell attachment or growth on the polysulfone+ fibers.

5.2 Verification of Cell Assembly and Culture Methods

The cell assembly and culture methods were verified using Fluent and other testing methods explained in this section. In order to verify this aspect of the bioreactor, the longitudinal flow, used for dynamic cell culture, and the vacuum force, used for cell seeding and assembly, were analyzed.

First the longitudinal flow field used for cell culturing was verified. The flow field of the cartridge was modeled in 2D using Fluent as shown in Fig. 5.10. As expected, the velocities greatly increase when entering the fibers because of the drastic change in area. Therefore it is necessary to determine an appropriate pump setting (inlet velocity) which will not disturb the growth of tissue.

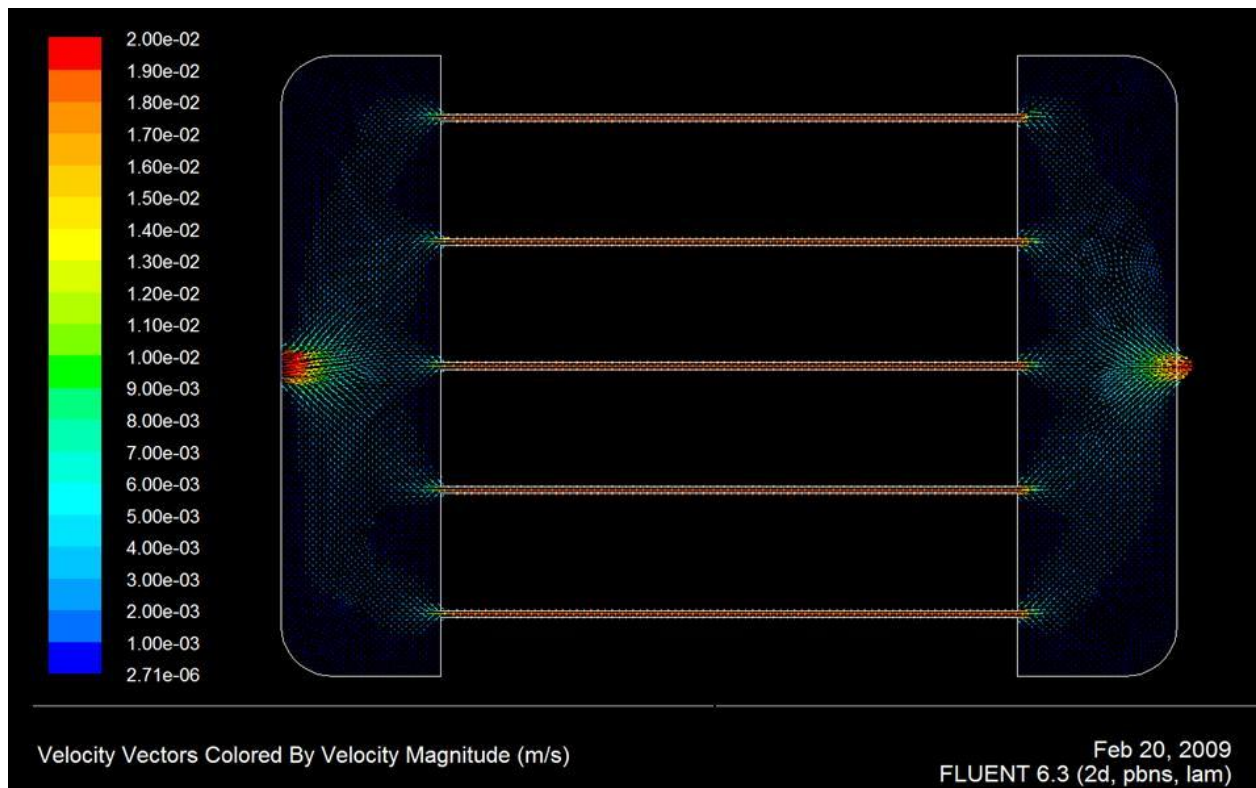


Figure 5.10: Fluent 2-D

Accordingly, this model allows a user to manipulate the inlet velocities in order to determine their respective fiber velocities so that an appropriate pump setting can be chosen. The pump flow rate was also determined based on the FiberCell manual which suggests flow rates of 1-4 ml/min per fiber during cell culture. The cartridge design proposed utilizes 5 fibers, so flow rates of 5-20 ml/min would be required to facilitate cell

culture in a single cartridge. Therefore this range of flow rates was tested using Fig. 5.10 above to determine the range of fiber velocities this would create.

The Fisher Scientific variable flow peristaltic pump model number 3389 selected for use in the bioreactor can produce flow rates of 4.0-600 ml/min. Flow tests using water were performed to verify the pump output and the actual range of flow rates achieved through 4 feet of 3/16th inch silicon tubing were 2-480 ml/min. The 4 feet of tubing was used to approximate the resistance of the entire bioreactor. Since each cartridge would require 5-20 ml/min during culture, the chosen pump has the additional capacity to supply several cartridges at once, and it could also supply cartridge designs with greater numbers of fibers.

The vacuum force through the wall of the fibers, used for cell seeding and assembly, was also verified using Fluent. Fig. 5.11 represents the flow around each fiber when the vacuum force is applied. The inlet velocity was set to 5×10^{-6} m/s and there was a negative pressure in the fibers of 5mmHg representing the vacuum force. For this model the inlets represent the access points where the cell solution would be injected for cell seeding. As expected the two ports as inlets on either side have the fastest velocity in comparison to the rest of the velocity vectors.

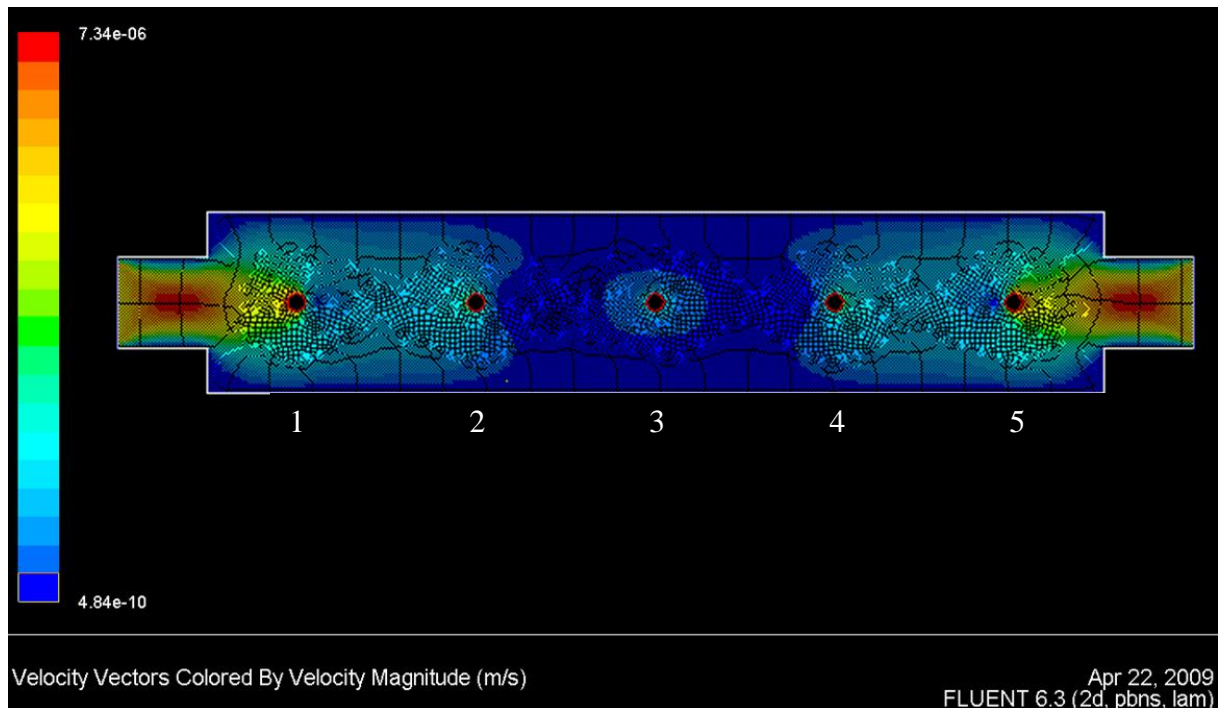


Figure 5.11: Flow Around Each Fiber

Figs. 5.12 and 5.13 depict the portion of the fibers which are directly under the inlet points (fibers labeled 1 and 5). Close to the inlet, there is a clear inequality of velocities circumferentially. However this 2D representation does not show the flow over the length of the fiber and therefore this extreme difference (approximately 6.8×10^{-6} to 1.1×10^{-6}) would not be experienced by the whole fiber. Fig. 5.12 and 5.13, depict fibers 1 and 5, shows some turbulence around the circular mandrel however when considering the magnitude of the velocities the turbulence is negligible.

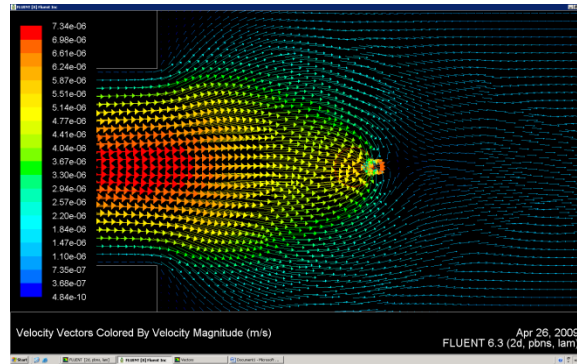


Figure 5.12: Fiber 1

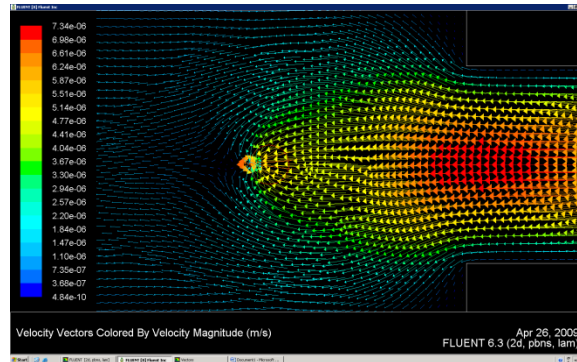


Figure 5.13: Fiber 5

Fig. 5.14, fibers 2 and 4, shows velocities which approach more circumferential symmetry. The inner side of the fiber, that which is opposite of the inlets, once again has slower velocities, but the range of magnitudes is much smaller, approximately 3×10^{-6} to 0.5×10^{-6} . The inlet flow is still influencing the overall unequal velocities, but the flow is slow enough that there is no turbulence around the cylindrical mandrel.

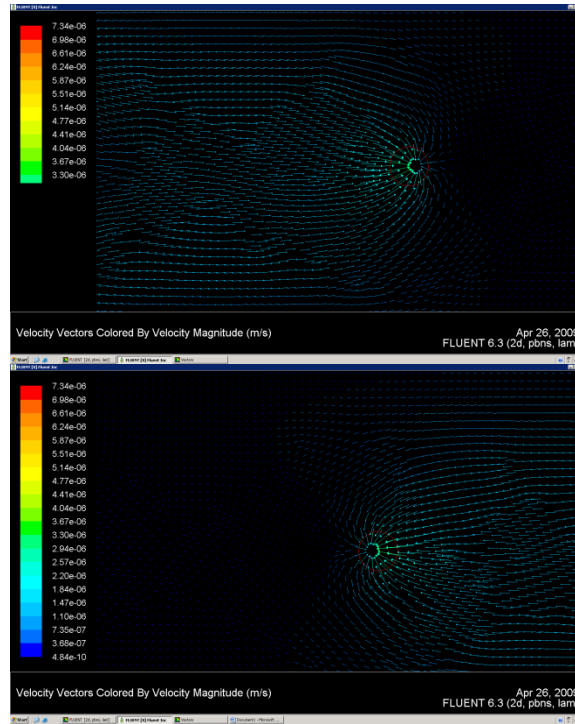


Figure 5.14: Fibers 2 and 4

Finally, Fig. 5.15 showed the center fiber, fiber 3. Since it is located in the center of the cartridge, the flows coming in from the ports are symmetrical on both sides, allowing for the uniform velocities seen below.

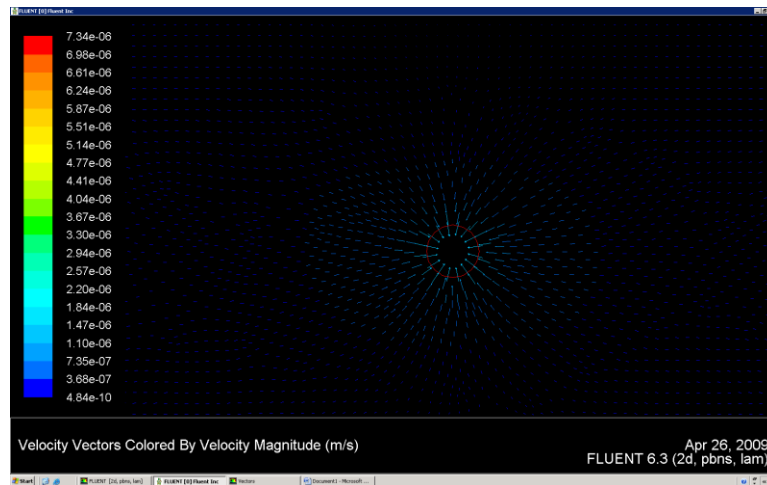


Figure 5.15: Fiber 3

Fig. 5.15, above, depicted the ideal scenario, that cells would be drawn uniformly around the circumference. However, because the pores allow for the media to pass through, but not cells, the imbalances would fix themselves. Cells are pulled to the mandrel, and the suction through the pores initially holds the cells in place. This action

blocks those pores and the open ones will have a stronger force since the same negative pressure will have less inlets. Therefore, the fibers that have uneven velocity distributions will change as the available pores decrease.

The above diagrams only show perspective of the flow around the fibers. It is also important to note the flow over the length of the fiber. Fig. 5.15 shows flow entering from two inlets, once again representing the access points where the cell solution is injection for cell seeding. As can be seen, the velocity decreases outside of the ports because of the change in area and spreads over the length of the fiber (shown in red). The same principle explained above applies here: as cells are seeded the remaining pores will draw the cells over the unseeded sections.

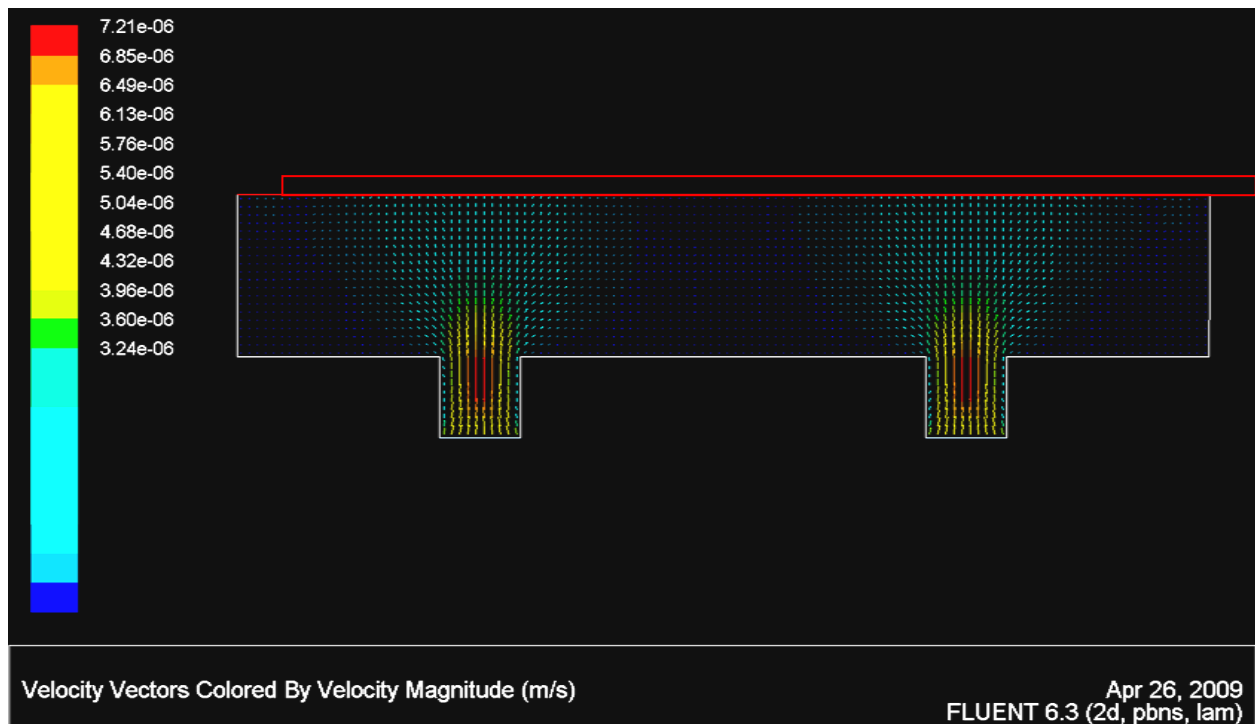


Figure 5.16: Flow Through Two Inlets

The pump was also tested to ensure that it was capable of producing flow in through the walls of the fibers in order to create a vacuum for cell seeding. The fibers chosen for use as mandrels were designed to be used in bioreactors where longitudinal flow through the fibers is all that is required for successful cell culture. The vacuum seeding of cells onto these fibers in the proposed bioreactor would require the temporary creation of an alternate flow during the cell-seeding period. The ability to create flow through the fibers was examined using a syringe pump and a pressure transducer. The

original Fibercell cartridge was connected to the syringe pump and a pressure transducer so that the pressures required to achieve flow through the walls of the fibers could be recorded. Flow rates of from 0.01-4.0 ml/min were achieved through each 5 cm of fiber with pressures of less than 5mm mercury. The actual flow rates used for vacuum seeding would ultimately be determined experimentally and through modeling of the flow and forces created at the surface of the fibers.

5.3 Verification of Housing to Culture and Monitor Cells

The housing to culture and monitor the cells was verified using Fluent and visualization testing. First Fluent was used to verify that the cartridge design allowed for uniform flow through each fiber by modeling the flow field of the cartridge design. Fig. 5.17 shows a 2D model of the longitudinal flow. The flow was assumed to be laminar and a range of velocities was tested.

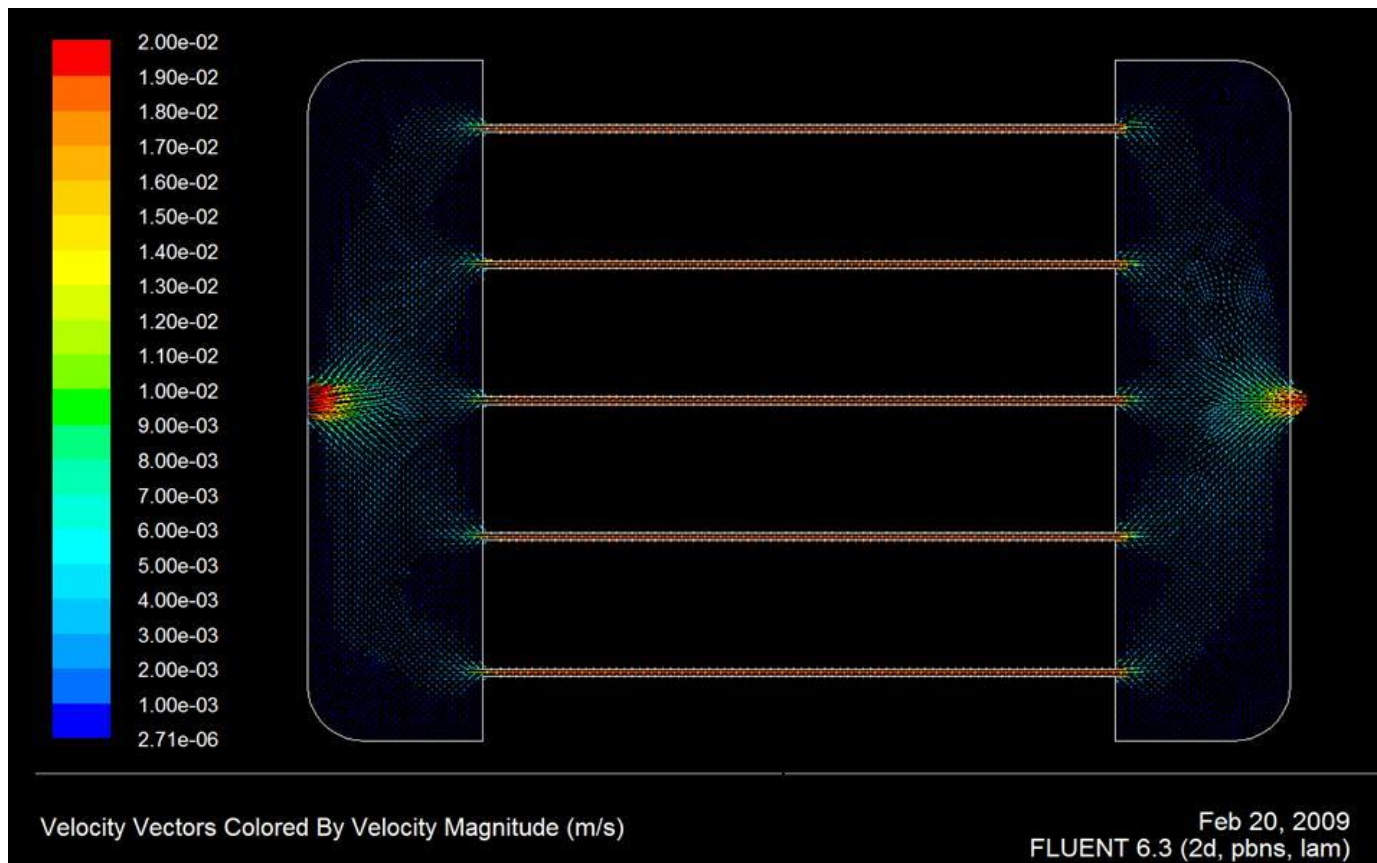


Figure 5.17: Flow Through Each Fiber

As can be seen in Fig. 5.16 above the flow evenly distributes through each fiber which verifying that the cartridge design allows for uniform flow through each fiber.

In order to verify the design of the cartridge the team needed to determine a cartridge width that would allow for visualization, using a microscope, of the polysulfone+ fibers without their removal from the cartridge. However the width of the cartridge was also determined based on the volume of media that could fit in it. As can be seen in Fig. 5.18 media, which is pumped through the fibers, does not fill the extra capillary space of the cartridge and therefore media must be manually injected into this space and manually changed based on its glucose levels.

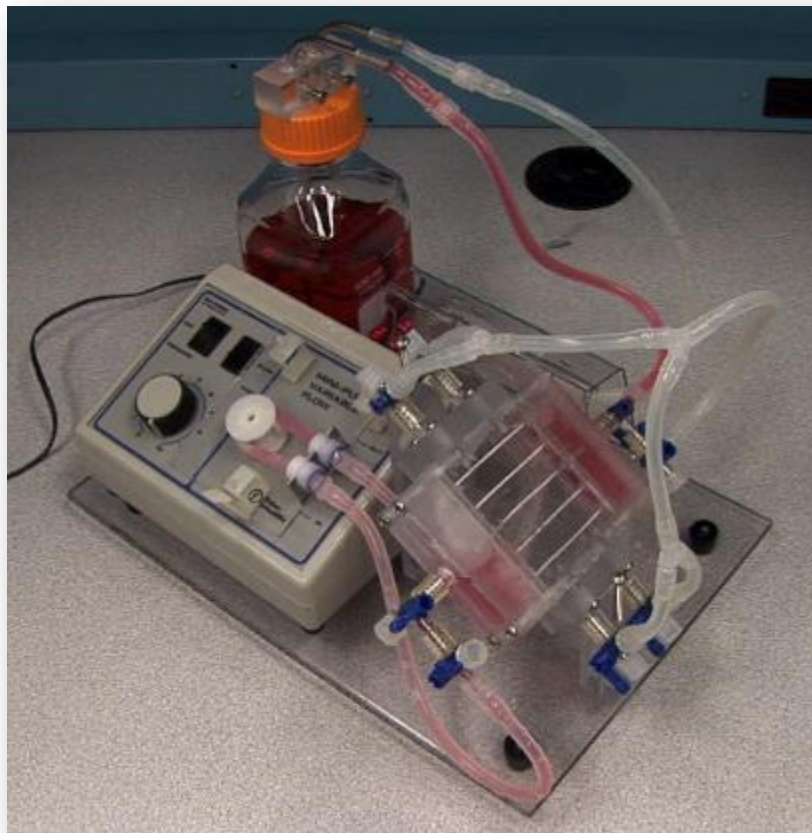


Figure 5 18: Bioreactor with Media

Thus it is important that a sufficient volume of media is contained in the extra capillary space so that glucose and oxygen levels remain sufficiently high between media changes. The extra capillary space of the FiberCell cartridge is approximately 27 cm^3 . In order to decrease manual manipulation and to ease the manufacturing process, an extra

capillary space of approximately 41 cm^3 was chosen. In relation to the extra capillary space of the Fibercell cartridges and in proportion to the number and length of fibers, this about 1.5 times the Fibercell volume. This resulted in a cartridge width of 1.27cm. This width was therefore tested under a microscope to ensure that the fibers could be put in focus and observed without removal from the cartridge.

6.0 Final Design and Validation

The construction of the prototype and testing was essential to begin to determine whether or not the design choice met all of the objectives and could be used to produce small-diameter vascular grafts. The following sections describe in detail the construction and verification of the bioreactor design choices. All materials used are listed in Appendix E, including prices, details of what was purchased and the company the products were purchased from.

6.1 Construction of the Bioreactor

The components of the bioreactor, pictured in Fig. 6.1, were obtained and connected using silicone tubing. Each component contributed to the function of the whole bioreactor, a media reservoir, a pump to move the fluid, a gas exchange tubing coil, and the cartridge to house the fibers and allow cell seeding and culture.

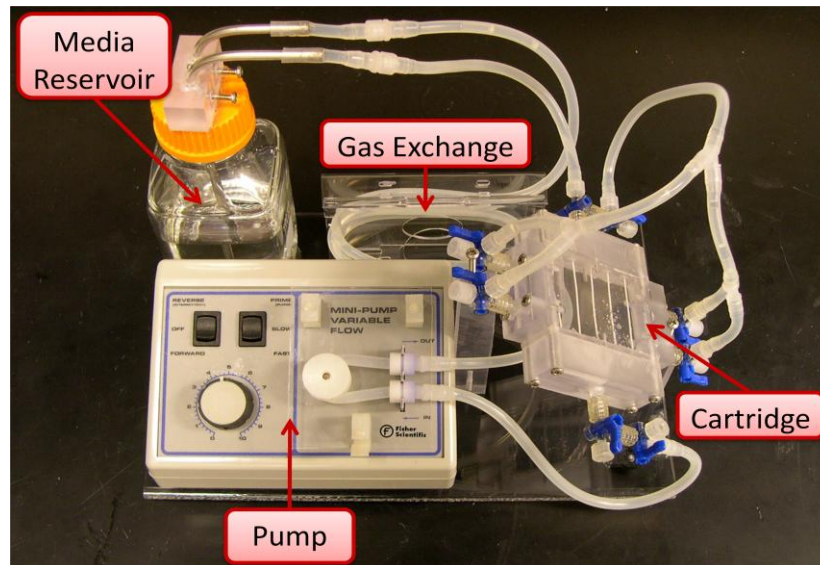


Figure 6.1: Completed Bioreactor

The CAD file for the cartridge was imported into GibbsCAM software in order to generate the CNC code required to machine the polycarbonate components of the cartridge. The tool choices, tool paths, and fixturing strategies were all discussed and planned with the aid of Neil Whitehouse, who runs the shop at WPI. The majority of the fabrication was done in the machine shop at WPI and the remaining fabrication was

completed in the MQP lab. The overall dimensioning of the thicker components was performed on manual mills and the thinner components were done completely on the CNC machine.

Once all the polycarbonate pieces were machined, a belt sander was used to correct edges that were not completely surfaced by the CNC machine, due to the thinner pieces falling out of the tool path before the last few centimeters were cut. A razor blade and knife were used to remove excess material from all edges and holes. Enough parts to assemble two complete cartridges were machined so that if any parts were damaged during fabrication there were spare parts to be used. As no parts were damaged during fabrication, two cartridges were assembled. Each cartridge fabricated consisted of two end caps and spacers (which together created a manifold). Fig. 6.2 and 6.3 show a spacer with holes for the ends of the hollow fiber mandrels in the center leaning against the cartridge housing, and the end cap to the right. Two 5.1 x 7.6 x 0.6 cm thick pieces made up the front and back of the cartridge housing and two 5.1 cm long T shaped blocks made up the top and bottom of the housing. The two access ports in each top and bottom T block and one port in each end cap were drilled and tapped for 10-32 threaded luer fittings.

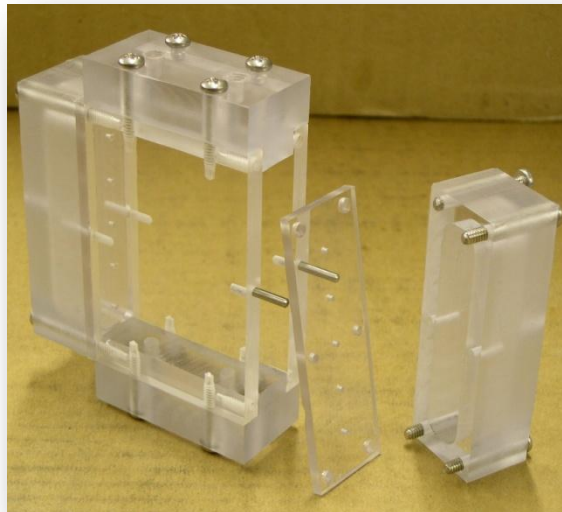


Figure 6.2: Disassembled Cartridge

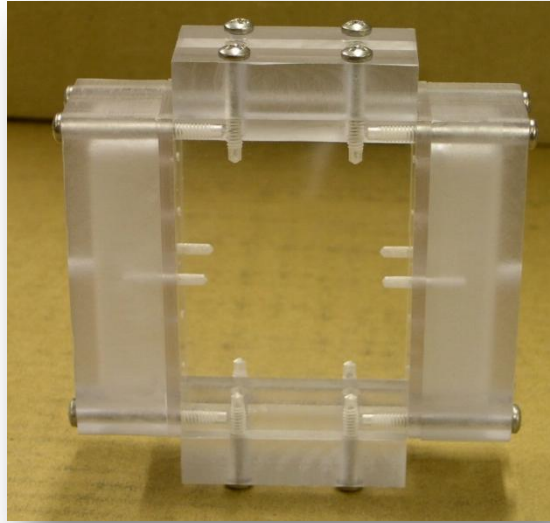


Figure 6.3: Assembled Cartridge

The hardware used to fasten the cartridge components together consisted of stainless steel 8-32 button head screws and 3/32 stainless steel dowel pins. The holes were tapped by hand and then the cartridges were test assembled to verify fitment of all components. The access ports were fitted with 10-32 luer fittings and then 4-way polycarbonate luer valves were attached to each fitting (Fig. 6.4).

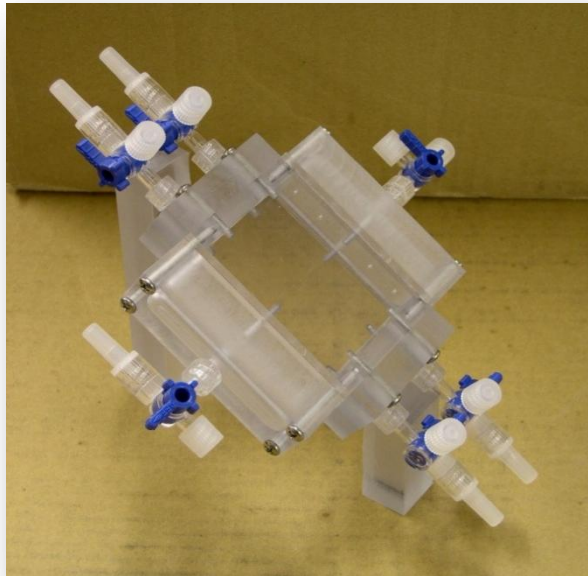


Figure 6.4: Cartridge with Luer Fittings

The two media reservoirs were created by drilling two holes in the caps of 500 ml pyrex bottles and inserting two 3/16th pieces of stainless steel tubing through the holes. The tubing was bent with a small hand held tubing bender to facilitate silicon tubing attachment and to reduce bending of silicon tubing when connected to the entire bioreactor (Fig. 6.5).



Figure 6.5: Media Reservoir

A platform was then fabricated that would hold all the components of the bioreactor together so that the entire assembly could be moved easily in and out of the incubator and flow hood. Fig. 6.1 incorporated the main components of the bioreactor and was attached with silicon tubing.

6.2 Validation of Final Design

In order to validate that the final design of the bioreactor worked as it was intended, flow testing using colored dye and Fluent was used to verify the final design, as described in Chapter 5.0.

6.2.1 Flow Testing Using Colored Dye

The bioreactor was tested in both the longitudinal flow path and in the vacuum seeding flow path with colored dye, to visually verify flow. The reservoir was filled with red dye and the valves were first adjusted to the settings required for longitudinal flow. The pump was set to the priming speed setting and red dye was drawn

out of the reservoir, through the gas exchange coil, longitudinally through the fibers and cartridge, through the pump, and back into the reservoir as in Fig. 6.6. The bioreactor functioned as expected in the longitudinal flow path and the red dye allowed a quick and simple qualitative verification of this.

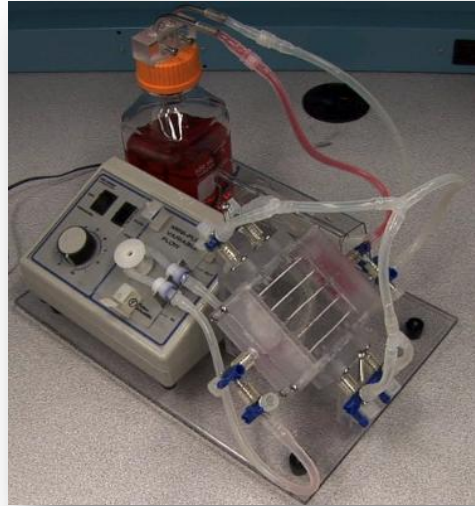


Figure 6.6: Dye Simulation of Longitudinal Flow Path

The vacuum seeding flow path was tested next, for which blue dye was used. The valves were adjusted to the settings for vacuum seeding, but instead of having flow into the cartridge through two ports, one port was tested at a time. The red dye from the first test was still present in the tubing and reservoir, but had not yet diffused through the fibers into the extra capillary space, which remained clear. A syringe with blue dye to simulate the cell solution that would be injected into the cartridge during vacuum seeding was connected to one of the luer valves on the cartridge. At the same time, another empty syringe was connected to the valve diagonally opposite as seen in Fig. 6.7.

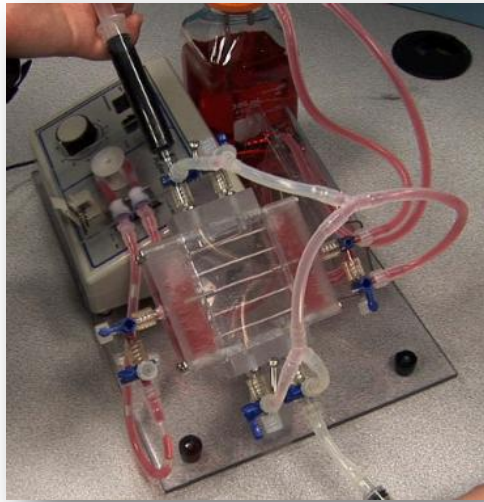


Figure 6.7: Cell Seeding Using Dye Simulation

The pump was turned on at the same time the syringe with blue dye was injected, slowly, into the cartridge. The red dye was seen coming in the lower port on the cartridge and the blue dye, simulating the cell solution, was seen coming in the opposite port, Fig. 6.8. The other syringe was slowly expanded to absorb the fluid displaced by the blue dye solution which was being injected into the extra capillary space. The valves were then adjusted so that the other port into the cartridge could be tested. The tests showed that the vacuum seeding pathways of the bioreactor also performed as expected. The blue dye representing the cell solution was also injected into the cartridge and drawn in through the walls of the fibers, and then out the end cap/manifold of the cartridge.

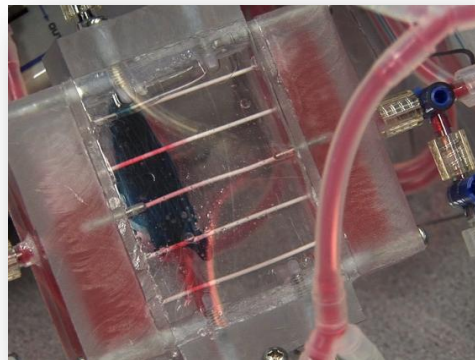


Figure 6.8: Vacuum Seeding Flow Path

7.0 Discussion

Each chosen aspect to fulfill the bioreactor functions are discussed in this chapter. The way the chosen design affects outside factors is also discussed briefly in this chapter to better understand the impact of it.

7.1 Bioreactor Design

The cartridge was fabricated with less than five dollars worth of polycarbonate and stainless steel hardware. Six hours of CNC machine time was required to make all the components for a single cartridge, mostly due to set up and part fixturing. This could be reduced to well under an hour if done by professional machinists and dedicated fixtures were designed for each component.

7.1.1 Mandrel Choice

The polysulfone fibers chosen for use as mandrels demonstrated excellent flow characteristics when evaluated for vacuum cell seeding suitability. The pressures required to achieve relatively high flow rates through the fiber walls were found to be less than 5mmHg. It is likely that the ultimate flow rates determined through experimentation to be appropriate for cell seeding will produce pressures of much less than this. Tests were completed to verify even pore distribution, by forcing air out through the walls of the fibers, while submerged in water, which resulted in uniform distribution of pores across the fibers surface. This validates that uniform vacuum seeding of the grafts should occur due to even pore distribution on the surface of the fibers.

7.1.2 Cartridge Shape

The cartridge shape did ultimately allow for visualization of all fibers through a light microscope from a single perspective. The challenge that remains is finding an appropriate means to access cell growth on the fibers during culture. The fibers exhibited a moderate degree of auto-fluorescence; however software could allow the baseline

fluorescence to be removed so that a measurable signal from the cells could be viewed. Once an appropriate cell tracking technology is found, the cartridge shape will allow for easy monitoring of the grafts during culture due to the linear fiber array, the flat faces of the cartridge, and the excellent optical clarity of the polycarbonate.

7.1.3 Vacuum and Cell Seeding

In general, when cells attach to the fibers, the pores that attracted those cells will be covered, leaving the uncovered pores more able to attract cells. This way even with uneven distribution over the fiber circumference, complete coverage is possible.

Cell seeding of the cartridge will be done using Fibercells recommendations of inoculating enough cells to achieve 50% coverage of the fibers assuming a 60-80% attachment rate. This would amount to 1-2 million cells per cartridge, since each cartridge has 5 fibers with total fiber surface area of 12.3 square centimeters.

Future research will focus on characterization of the fibers with SEM so that the forces experienced by the cells at the surface of the fiber during vacuum seeding can be more accurately modeled. These models will be correlated with experimental outcomes resulting from seeding and culturing the grafts so that optimized cell seeding protocols can be developed. From these models and experiments new insights into cellular responses to mechanical forces will be gained.

7.2 Economic Impact

If the bioreactor were ultimately successful in generating vascular grafts that could gain FDA approval for clinical use, the economic impact could be tremendous. The money saved by avoiding the harvest of autologous vessels, and treating the complications associated with synthetic grafts, would likely more than offset the costs of generating the tissue engineered vascular grafts.

The production of this device would also affect those researchers interested in cardiovascular disease and could reduce or eliminate the need to sacrifice animals for the advancement of their research. The bioreactor can be used as an ex-vivo vascular model by co-culturing SMCs with an endothelial monolayer. In this way a cost effective model

using human cells could be easily and readily available to further research into cardiovascular disease.

There are also potential applications in all of tissue engineering, since the artificial capillary cell culture techniques used here to produce the vascular grafts could be applied to other tissues as well. The tissue engineered vascular grafts themselves could be used in order to vascularize other tissue engineered tissues or organs as they are cultured, or when implanted into patients.

7.3 Environmental Impact

If this device were used in clinical applications, the cartridge would most likely be replaced for each patient due to the lower cost of replacing the polycarbonate cartridge versus the labor to resterilize the components. The minimal labor required to resterilize the cartridge however, would allow an environmentally conscious company to reuse the cartridges with little increase in overall production costs.

7.4 Societal Influence

The successful production of tissue engineered vascular grafts could benefit a large cross section of society, both in quality and quantity of life. Patients suffering from cardiovascular disease would be able to use their own cells to produce tissue that could replace their diseased vascular tissue. One in four deaths is caused by cardiovascular disease and having this option could save many lives.

7.5 Political Ramifications

Many countries believe that tissue engineering is not socially acceptable. Producing a bioreactor that can grow tissue is looked down upon by many religions. Although some countries would be against the use of this device, it could benefit many patients and researchers around the world.

7.6 Ethical Concern

With most tissue engineering advances, there are ethical concerns involved. Although a replacement blood vessel has the potential to save many lives, some feel that growing tissue is not acceptable. While there are options, the device would make it easier and cause fewer complications than options available today.

7.7 Health and Safety Issues

The device has the potential to improve the health of patients suffering from cardiovascular disease and is an alternative to methods that have been shown to cause side effects. Replacing diseased tissue can be done by harvesting blood vessels from other parts of the body which requires multiple surgeries and often leads to suffering where the tissue was harvested from. Synthetic grafts often lead to compliance mismatch and cause thrombosis. Currently, the method of growing tissue is time consuming, which may be more time than the patient might have. Replacing diseased tissue with tissue engineered vessels made from the patient's own cells decreases the risk of immune rejection that may result from using donor tissue.

7.8 Manufacturability

The device could be mass produced using the specifications and machining files that were developed. The device was designed with reproducibility in mind in that it is inexpensive and easy to manufacture. Cell harvesting and tissue growth would have to be done by a professional but with proper use of the device; the tissue would be easily produced. If the device were used to produce vascular grafts to be implanted in the body, a new cartridge could be used for each patient. Using the same set-up of the bioreactor with each patient would allow the user to only replace the cartridge, thereby eliminating steps from the process.

7.9 Sustainability

Although this device does not directly use energy, it requires the use of an incubator and vacuum pump. Growing tissue would require more energy than conventional methods of harvesting tissue from donors. The device could easily be used in an incubator that is environmentally friendly and vacuum source that uses less energy.

8.0 Conclusions and Recommendations

Although time constraints prevented tissue culture using the final bioreactor design, each aspect of the bioreactor was validated: the polysulfone+ fibers used as a structure to support cell growth, the pump and vacuum used for cell culture and seeding, and the cartridge design used to house and monitor cell growth. This provides a proof-of-concept suggesting that the bioreactor could be used to generate vascular grafts.

8.1 Design Choice

The design of a novel bioreactor which allows cells to self assemble into tubular constructs was accomplished. The vacuum and flow patterns shown in Fluent, in conjunction with testing of the fibers, leads to the conclusion that cells could assemble uniformly around the mandrels. The bioreactor design is also beneficial because it allows for changes in fiber diameter and number based on the application. Additionally the bioreactor allows for a sterile environment where mechanical conditioning and testing can be performed in the future. This could be done in the case that a degradable mandrel is used, as the pump system could be used at different pressures through the vessels. Ultimately the design must be validated by seeding the cartridge with cells.

8.2 Future Recommendations

The team recommends that a method to visualize cell growth on the fibers should be a focus of future studies. The flow field of the cartridge should be modeled in 3D using Fluent in order to completely analyze the fluid dynamics of the cartridge. Cells should also be modeled in the cartridge using Fluent to determine how the vacuum force would affect the cells. The cartridge design could be further optimized to ease manufacturing and combine cartridge parts so there is less chance of leakage.

The fiber could be changed to find ideal porosities for culture and vacuum seeding or could be manufactured based on desired specification using electrospinning. A fiber material that degrades over a specified period of time would be ideal eliminating

any vascular graft manipulation. Additionally, a degradable mandrel would more easily allow resulting vascular grafts to be tested without removal from the cartridge. The development and manufacture of this new mandrel would in itself be a huge undertaking, requiring investigation of different polymers and degradation rates that allow for enough ECM production before the support disappears.

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Glossary

Anastomosis – the connection between vascular structures.

Autologous – transplanted tissue from the same patient.

Coronary Artery Bypass Graft – Surgery performed to correct arterial blockages in the heart by implanting a graft, restoring flow distal to the blockage.

Coronary Heart Disease – Conditions affecting the vasculature in the heart.

Electrospun – the production of micro- or nano-scale fibers with electrical charges.

Exogenous scaffolds – an externally produced structure for tissue culture to incorporate into.

Graft – a transplanted vessel.

Hemocytometer – a device that counts cells.

Hemodynamic loading – the forces against a vessel from blood movement.

Human Dermal Fibroblasts – cells derived from the dermis of a human, can be obtained by skin biopsy.

In Vitro – experimentation within a controlled environment.

In Vivo – experimentation within a living organism.

Internal Mammary Artery – also known as the internal thoracic artery, supplying blood to the anterior chest wall and breasts.

Mandrel – a structure used for shaping.

Media – also known as cell culture media, is a liquid or gel designed to support cell growth.

Myocardial Infarction – commonly known as a heart attack, occurs when blood flow in the heart is interrupted.

Polysulfone+ – a thermoplastic polymer that allows for proteins to bond.

Saphenous Vein – vein located in the superficial posterior leg.

Smooth Muscle Cells – involuntary, non-striated muscle cells.

Thrombosis – the development of a blood clot within a vessel.

Appendixes

APPENDIX A: Pairwise Comparison Charts

BIOREACTOR	<i>Continuous nutrient delivery</i>	<i>Outputs tubular construct</i>	<i>Easy to use</i>	<i>Low cost</i>	<i>Safe for user</i>	<i>Fast result times</i>	<i>Multiple cultures</i>	SCORE	Weight	Percent
<i>Continuous nutrient delivery</i>		0	0.5	1	0	0	0.5	2	0.103	10.26
<i>Outputs tubular construct</i>	1		1	1	0	1	1	5	0.256	25.64
<i>Easy to use</i>	1	0		1	0	0	1	3	0.154	15.38
<i>Low cost</i>	0	0	0		0	0	0	0	0.000	0.00
<i>Safe for user</i>	1	1	1	1		1	1	6	0.308	30.77
<i>Fast graft generation</i>	1	0	0.5	1	0		1	3.5	0.179	17.95
<i>Multiple cultures</i>	0	0	0	0	0	0		0	0.000	0.00

Vascular Grafts

VASCULAR GRAFTS	<i>Suturable</i>	<i>Easy to handle</i>	<i>High burst pressure</i>	<i>Self generated ECM</i>	<i>Fast growth rates</i>	<i>Reproducible</i>	<i>Biocompatible</i>	SCORE	Weight	Percent
<i>Suturable</i>		1	0	0	0	0	1	2	0.100	10.00
<i>Easy to handle</i>	0		1	0	0	0	1	2	0.100	10.00
<i>Appropriate burst pressures</i>	1	0		0	0	0	0.5	1.5	0.075	7.50
<i>Self generated ECM</i>	1	1	1		0.5	0.5	1	5	0.250	25.00
<i>Fast growth rates</i>	1	1	1	0		0	1	4	0.200	20.00
<i>Reproducible</i>	1	1	1	0	0.5		1	4.5	0.225	22.50
<i>Biocompatible</i>	1	0	0	0	0	0		1	0.050	5.00

APPENDIX B: Functions/Mean Morphological Chart

Functions	Means			
Creates/Supports tubular construct	Mechanical Mandrel	Tubular Dye	Tubular Mandrel	Negative Space Mold
Allow removal of intact tubular construct	Mechanical Mandrel	Material Properties	Material Coatings	Chemical Solutions
Facilitate cell seeding	Pump/ Vacuum	Material Properties	Material Coatings	Rotation
Promote cellular growth	Pump/ Vacuum	Nutrient Permeable Materials	Media Composition	Growth Factors
Promote cell adhesion	Pump/ Vacuum	Material Properties	Material Coatings	Rotation
Allow nutrient diffusion/ gas exchange	Pump/ Vacuum	Permeable Materials		Rotation
Allow for visualization of fibers	fibers around perimeter of housing	flat housing (fibers in line)	clear housing	
Housing for fibers	cylindrical	rectangular	flat	
Fixation of Fibers	o-rings	silicone sealant		
Vacuum adheres cells to mandrel	pump flow from outside surface of mandrel to inside surface			
Closed off housing	reverse threaded cap	clamped cap	pop off cap	
Fibers are easily removed	reverse threaded cap	both ends are not permanently fixed	one end of housing permanently fixed/ one not	

APPENDIX C: Decision Matrixes

Mandrel Type

Decision Matrix				
	Mechanical Mandrel	Tubular Dye	Tubular Mandrel	Negative Space Mold
Objectives				
Reproducible	2	2	2	2
Tubular Vascular Graft	2	2	2	2
Multiple Cultures	2	2	2	2
Fast production time	2	2	2	2
Low cost	0	2	2	1
Easy to use	1	2	2	1
Constraints				
Time	1	2	2	2
Budget	0	2	2	2
No exogenous scaffold	2	2	2	2
Fits in incubator	2	2	2	2
Sterilizable	2	2	2	2
Functions				
Creates/Supports tubular construct	2	2	2	2
Facilitate cell seeding	1	2	1	2
Promote cellular growth	1	2	2	1
Promote cell adhesion	1	1	1	2
Allow removal of intact tubular construct	2	1	1	1
Allow nutrient diffusion/gas exchange	1	2	2	1
Total	24	32	31	29

Cell Seeding Method

Decision Matrix			
	Manual	Pump/Vacuum	Rotation
Objectives			
Reproducible	1	2	2
Tubular Vascular Graft	1	2	2
Multiple Cultures	2	2	2
Fast production time	1	2	2
Low cost	2	1	1
Easy to use	0	2	2
Constraints			
Time	1	2	2
Budget	2	1	1
No exogenous scaffold	2	2	2
Fits in incubator	2	2	2
Sterilizable	2	1	1
Functions			
Creates/Supports tubular construct	1	2	2
Facilitate cell seeding	0	2	2
Promote cellular growth	0	2	2
Promote cell adhesion	0	2	0
Allow removal of intact tubular construct	2	2	2
Allow nutrient diffusion/gas exchange	1	2	2
Total	20	31	29

Cartridge Shape

Decision Matrix: Cartridge Shape				
	Cylindrical	Rectangular	Flat	Triangular
Objectives				
Fibers can be easily removed/ installed	2	2	2	2
Cells are seeded directly into cartridge	2	2	2	2
Easy removal of unattached cells	2	2	2	2
Fits 12 fibers	2	2	1	2
Functions				
Allow for visualization of fibers	0	2	2	2
Housing for fibers	2	2	2	2
Housing should be closed	2	2	2	2
Fixation of fibers	2	2	2	2
Vacuum adheres cells to mandrel	2	2	2	2
Facilitate cell dispersion during seeding	2	2	0	2
Constraints				
No cell adherence	2	2	2	2
Total	20	22	19	22

APPENDIX D: Protocols

Harvest of Fibers from Cartridge

Materials- Steel hacksaw blade

Surgical scissors

Sterilizable containers for fibers (10-20)

(Aluminum foil?)

steel probe/large sewing needle (open fiber end after cutting with scissors)

Lengths of stainless steel welding rod to gauge fiber lengths to cut

Labels for fiber lengths/numbers in each container

Tape/caps to cover fittings after removal of cartridge

Stainless steel welding rods cut for use as length gauges

2 Male luer lock plugs

- 1.) sterilize containers, scissors, saw blade, welding rod, probe/needle, razor blades,
- 2.) Place all sterilized materials in hood.
- 3.) Open fiber cell cartridge assembly bag in hood and remove cartridge by unscrewing luer lock fittings at each end and immediately plug each luer lock with sterile male plugs. Place assembly minus cartridge back in bag and reseal with tape.
- 4.) Cut off each end of cartridge flush with inside of edge of urethane end disk with either saw blade or razor blade (razor blade will reduce risk of particles on fibers).
- 5.) Cut fibers in half, then cut halves into shorter lengths for testing if required. Use welding rod gauges to aid in cutting to required lengths. Use probe/needle to verify that ends of fibers are fully open and round.
- 6.) Place fibers in containers in groups appropriate for experimental method so that all fibers in each container will be used at the same time.
- 7.) Close/seal containers and label for storage.

Video Camera resolution/suitability test

Materials: Hi-def Video camera

 Tripod

 Scale drawing of vascular graft dimensions and wall thickness.

Procedure:

1.) test focal range and resolution of camera by filming scale drawing as close as possible.

2.) evaluate image, crop, magnify, down convert .

Polycarbonate Optics tests

Materials:

Different thickness polycarbonate sheets

Microscope

camera

video camera

fiber

Purpose: Establish thickness that can be used for viewing

Procedure: Position fiber at multiple distances away from microscope and subjectively decide what the furthest distance the fiber can be from the base of the microscope and still be visible. These steps should be repeated with the camera and video camera.

Bioreactor

Materials: media bottle
 Reservoir
 cap
 Luer locks/fittings that match fibercell components
 Syringes as described in fibercell protocol
 Tubing of equal size to fiber cell set up
 Tubing for pump compression zone

Procedure:

1. Drill two .3 inch holes in cap of an autoclavable media.
2. Using silicone glue, glue 0.25 ID tubing to each of the holes.
 - a. One tube will directly attached to the pump
 - b. One tube will be the outlet of the cartridge

Using Trypan Blue for Cell Count

1. Add 40µL of Trypan Blue to

Preparation of the CellTracker

1. Allow CellTracker to warm to room temperature which was stored below 21°C.
2. Dissolve with DMSO to concentration of 10mM. When dissolving 1 mg of product with a 464.86 MW:
$$0.001\text{g} \times (1\text{mole}/464.86\text{g}) = 2.1512 \times 10^{-6} \text{ mole}$$
$$0.01\text{m/L} = (2.1512 \times 10^{-6} \text{ m/ x L})$$
$$X = 0.000215\text{L} = 215 \mu\text{L of DMSO added to CellTracker}^*$$
3. Remove 10 µL of the working solution and place in 15mL centrifuge tube.
4. Dilute the stock solution to a working solution with a concentration of 5µM by adding 20mL of serum free media to the 50mL tube.
$$(5\text{mM})(0.01\text{mL}) = (0.005\text{mM})(x)$$
$$x = 20\text{mL (allows for ~20 aliquots of CellTracker)}$$
$$\text{(if adding 10}\mu\text{L then 20mL of serum free media should be added- allows for ~20 aliquots)}$$
5. Warm the working solution to 37°C.

*Step 2 was only performed once to create a stock solution of cell tracker. For each experiment involving CellTracker, the stock solution was warmed to room temperature and a working solution was made as explained in Steps 3-5.

Hanging Drop Experiment 1

Prepare 3 six well plates to be seeded using the hanging drop method as described in Section 5.1.1. Seed 13 wells with 280 μ L of a 10⁶ cell/mL media solution. One well will be used as a control. Seed three with the following protocol:

1. Using hemocytometer determine a cell count. Based on cell count prepare a cell solution of 10⁶cells/mL concentration. This way once the CellTracker is added it will be easy to resuspend the cells in the correct amount of fresh media without recounting
2. Centrifuge cells and aspirate remaining media
3. Resuspend cells in CellTracker working solution (refer to Preparation of CellTracker)
4. Incubate for 15-45 minutes
5. Centrifuge cells
6. Replace working solution with fresh media (same amount added to prepare 10⁶cells/mL concentration)
7. Incubate for 30 minutes
8. Resuspend cells
9. Add cell solution to 3 prepared hanging drop wells
10. Observe after 1hr and after 24 hrs

Every three days add CellTracker solution to 3 wells so that it is added to the last plates at day 15 using the following protocol:

1. Remove media
2. Add CellTracker working solution
3. Incubate cells for 15-45 minutes
4. Replace working solution with fresh media
5. Incubate cells for 30 minutes
6. Observe fibers immediately

Hanging Drop Experiment 2

1. Prepare 3 six well plates to be seeded using the hanging drop method as described in Section 5.1.1.
2. Seed 15 wells with 280 μ L of a 10^6 cell/mL media solution. The remaining well is used as a control but media should be added and changed as done with seeded wells.
3. After three days of culture, add CellTracker solution to 3 wells using the following protocol:
 - a. Remove media
 - b. Add CellTracker working solution
 - c. Incubate cells for 15-45 minutes
 - d. Replace working solution with fresh media
 - e. Incubate cells for 30 minutes
 - f. Observe fibers immediately
4. Continue adding CellTracker every three days until all wells have had CellTracker added to them using the protocol in Step 3.

V-Well Experiment

1. Prepare PDMS V-well as described in Section 5.1.2
2. Place six 2cm preconditioned polysulfone+ fibers in the wells (2 fibers in each well)
3. Add 1mL of 1.85×10^6 cells/mL concentration cell solution to each well
4. Allow the cells to seed onto the fibers for 1 hr
5. Aspirate remaining cell solution and add fresh media
6. After three day of cell culture add CellTracker working solution using the following protocol:
 - a. Remove media
 - b. Add CellTracker working solution
 - c. Incubate cells for 15-45 minutes
 - d. Replace working solution with fresh media
 - e. Incubate cells for 30 minutes
 - f. Observe fibers immediately

Cartridge Seeding Protocol

A. Preparation of Bioreactor

- a. Close the inlet and outlet of the cartridge to isolate it from the flow path.
- b. Flush ethanol through the cartridge for at least 1 minute using the syringes connected to the access points on the cartridge.
- c. Flush it with PBS twice.
- d. Open the inlet and outlet of the cartridge.
- e. Pump media through the bioreactor until the system is filled.
- f. Tilt the bioreactor to remove air bubbles and check for leaks.
- g. Close the inlet and outlet of the cartridge to isolate it from the flow path.
Ensure that there are no air bubbles or leaks in the cartridge.
- h. Pump the media through the system for 1 minute at maximum speed, check for leaks.
- i. Incubate the bioreactor filled with media for three days changing the media and pumping the media through every 24hrs.
- j. Before seeding, change the media.

B. Preparation of cells

- a. Trypsinize 80% confluent HDFs from two, 75cm² cell culture flasks and centrifuge.
- b. Aspirate media.
- c. Resuspend cells in fresh media and get cell count using a hemocytometer.
- d. Prepare a cell solution of 1.5×10^6 in 5 mL of media.

C. Seeding the bioreactor

- a. Isolate the cartridge from the flow path and add the cell solution using a syringe through one access point. A syringe is connected to the other access point. Both syringes will be used to flush the cell solution back and forth to ensure a uniform cell suspension throughout the cartridge.
- b. Ensure that the excess cell solution is distributed evenly between the syringes.
- c. Close one syringe access point and open one side of the cartridge.
- d. Add the remaining cell solution from that syringe.
- e. Repeat for the other syringe.
- f. Change from longitudinal flow path to vacuum flow path.
- g. Vacuum Seed for 2 hours.

APPENDIX E: Bill of Materials

Plastics Unlimited Inc.

<http://www.plasticsunlimitedinc.com/>

Product	Details
Polycarbonate sheet	1"thick
Polycarbonate sheet	1/4" thick
Polycarbonate sheet	1/8" thick
<i>drop offs/random size pieces</i>	<i>\$6.00</i>

Small Parts Inc.

<http://www.smallparts.com>

Part Number	Units	Details	Cost
XPHP-0632-12-50	50 / pack	3/4 inches (316 6-32 ssteel screws)	\$3.11
XPHP-0632-16-50	50 / pack	1 inches (316 6-32 ssteel screws)	\$3.49
PPHX-0632036-25	25 / pack	1 1/2 inches (316 6-32 ssteel screws)	\$3.26
DWXX-021-10	10 / pack	(ssteel 3/16 dowel pins)	\$7.40
A1-LXM021	10 / pack	(M luer to 10-32)	\$5.40
B1-LXF018	10 / pack	(F luer to 10-32)	\$4.50
FTLL250-6	25 / pack	(F leur to 3/16 barb)	\$6.67
MTLL250-6	25 / pack	(M leur to 3/16 barb)	\$7.50

Factor II Inc.

<http://www.factor2.com/>

Product #	Details	Cost
A-100-S	Type A Medical Adhesive Sterile	\$19.95

Harvard Apparatus

<http://www.harvardapparatus.com>

Product #	Details	Cost
722635	3-Way, FLL to MLL with Port Covers (50)	\$121.00

FiberCell Systems Inc.

<http://www.fibercellsystems.com>

Product #	Details	Cost
4300-C2025	Small Polysulfone Plus Cartridge 0.1µm	\$188.50